

Has Mrs Thatcher turned green?

The British prime minister has acknowledged that greenhouse gases are an environmental hazard. But how soon should something be done?

Too much will almost certainly be made of the declaration last week by Mrs Margaret Thatcher, the British prime minister, that the time may have come when "we have unwittingly begun a massive experiment with the system of the planet itself". What Thatcher did, at the end of an address to the Royal Society (see p.484), was to say that there had been an increase of greenhouse gases, that there is evidence of the depletion of the ozone layer above the Antarctic in the Antarctic spring and to remark on the prevalence of acid deposition, popularly known as acid rain. While much of what she had to say will be widely welcomed, and although Thatcher referred in what she had to say to other forms of pollution (old-fashioned and now-banished London smogs, for example), it is probably mistaken that she has been widely proclaimed a convert to green causes by a variety of newspapers. Speculation that her declaration, whatever its logical basis, is intended to strengthen the government's political position is probably well wide of the mark: the annual season of political conferences, now almost at an end, has shown that the government has little reason to fear its rivals.

It is particularly welcome that a senior politician should have gone out of her way to acknowledge that the greenhouse gases are potentially a serious problem. For much of the past year, it has been plain that the continuing accumulation of carbon dioxide needs to be taken seriously, preferably by planning to negotiate the international agreements that would be necessary to fend off unwanted climatic change. Although the international convention on the ozone layer, finally negotiated last year at Montreal, is a sign that it is possible to make modest progress even in circumstances in which individual nations' self-interests conflict with the international need, reaching an international convention on the greenhouse gases will be much more difficult. The chlorofluorocarbons whose manufacture is restricted by the Montreal convention are, after all, only marginally important to the economies of those who make and use them. The production of carbon dioxide, by contrast, is everybody's bread and butter.

Climatic change

Very little imagination is needed to tell what kinds of problems will arise. First, there could be no question of an outright and thus easily policed ban on the production of carbon dioxide, which would prevent the burning of wood in domestic fires as well as the use of fossil fuels in, say, electricity generating plants. The best that can be worked for is an agreement among sovereign states that their citizens will observe negotiated constraints in their production of greenhouse gases. But what constraints will apply to whom?

Will nations likely to suffer most from predicted climatic change be required to accept the tightest constraints? Will the quantities of carbon dioxide and other greenhouse gases that nations are permitted to release be fractions of what they at present release, thus linking them indirectly with Gross National Product or some other measure of wealth? What arrangements will there be to meet the inevitable demand from developing countries that their economic development must be disproportionately constrained by an agreement not to release greenhouse

gases at the earliest stages in their development? There is very little chance that any one of these issues will be quickly settled, which is why the sooner negotiations begin, the greater the chance that they will be completed before the ice-sheets begin to melt.

Paradox

Paradoxical though it may seem to the people who will have to conduct the negotiations, it is important to begin now on this daunting task although the scale on which the greenhouse effect is likely to affect the climate of the Earth, and the likely timetable for change, is still a matter for conjecture. This is a lot to ask of sober-sided governments, which are habituated to dealing only with the most immediate problems, and which have a dread of tackling questions that might be thought hypothetical. Thatcher herself, by the evidence of last week's speech, seems impressed that the past decade includes five of the warmest years in a century of meteorological records, and it is entirely possible that her opposite numbers elsewhere will be similarly impressed that global warming may already have begun. The snag is that, because the weather is statistical in character, the decade that lies ahead may include no such warm years, which may be taken by some as an excuse for postponing alarm about the prospect of climatic change. That would be disastrous, which is why it is important that everybody should understand that there are, at present, only imprecise calculations of how and when climatic change will become evident. Moreover, it is unlikely that the remaining uncertainties, especially those associated with the production of methane both artificially and naturally, can be cleared up by further research before the need for restraint has become overwhelming. Yet "sometime next century" remains as good a guess as "sometime next decade".

The greenhouse problem is distinctive because it entails as its end-product an irreversible phenomenon — the melting of the ice-sheets — whose consequences would be a general calamity, economically and otherwise a much more serious development than the climatic changes which may affect the decades more immediately ahead. This is why the problem lies to some extent outside the framework in which questions of the proper basis for regulating pollution are most rationally discussed. Thatcher noted last week that Britain and similar countries have become wiser in dealing with pollution with the passage of time, which is another way of saying that prosperous countries are those most able to afford the relative luxury of environmental protection. Moreover, environmental protection always entails the use of economic resources in some manner, which means that freedom from pollution is almost always a public purchase made by governments on behalf of their constituents. It is important that these truths should not be obscured by the greenhouse problem and whatever steps are now taken to fend it off. Fortunately, Thatcher's influence, much to be welcomed if it is her intention to dramatize the need for early international planning in this troublesome field, is likely to be exerted in the direction of rationality on more domestic issues — which is another reason why it would be premature for the greens too warmly to welcome her as a convert. □



United States back in space again

- Discovery completes perfect flight
- NASA free to look to the future
- International space station agreement signed

Washington

ON 29 September, the same day that the launch of the space shuttle Discovery marked the return of the United States into space after a forced hiatus of two and a half years, the first steps were taken towards establishing a permanent manned presence in space. At a ceremony in Washington five hours after the launch of Discovery, representatives of all the United States, Europe, Japan and Canada signed an agreement on the joint construction and operation of an international space station.

Discovery climbed smoothly into orbit only an hour and a half after its scheduled lift-off time. Winds at 40,000 feet were unexpectedly light, and the launch was put off until flight controllers were sure the trajectory would not be unduly affected. About 90 seconds before the end of the resumed countdown, an intended halt at 31 seconds was announced because of problems with cabin pressurization, but the difficulty was resolved, the countdown was not interrupted, and the shuttle left the ground at 11:37 a.m. An anxious silence was broken when the solid-fuel boosters were successfully discarded two minutes into the flight, and from then on Discovery sailed into its intended orbit. The main purpose of the mission, deploying the TDRS (Tracking and Data Relay Satellite), was accomplished only six hours after the launch. After release from the shuttle loading bay, TDRS was boosted into a geostationary orbit by its own two-stage rocket. The new satellite joins a similar one launched in 1983 to give the National Aeronautics and Space Administration (NASA) needed capability to communicate between ground and space. Many future orbital facilities, notably the Hubble Space Telescope, will rely on the TDRS system to pass data to Earth.

Discovery glided down to land at Edwards Air Force Base in California, arriving at 9.37 a.m. local time exactly as scheduled. The successful mission leaves NASA free to begin thinking more generally about the future in space.

The space station was first proposed as an element of US plans for space in President Ronald Reagan's 1984 State of the Union address, at which time he also emphasized his desire to seek international collaboration to build it. But although Japan, Canada and several members of the European Space Agency (ESA) expressed immediate interest,

negotiations over the past four years have been hampered by disputes over the kind of research, civil or military, that would be conducted on the space station, and by difficulties in establishing a consensus on sharing the costs and allocating use of the facilities. In the intergovernmental agreement signed last week, the participants settled who would provide what parts of the station and agreed to managerial procedures to run it. The United States will build the basic framework, supply living quarters and power resources, furnish its own laboratory, and build an independent polar-orbiting platform that will work in conjunction with

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REASONS

US return to space — Discovery lifts off from Kennedy Space Center last Thursday morning.

the space station. Canada's contribution is its Mobile Servicing System, a free-moving robotic elaboration of the manipulating arm it has provided for the shuttle. Japan and ESA will each build attached experimental modules, and ESA will also supply two free-flying vehicles for long-term experiments. Each member of the collaboration will get the use of all experimental facilities for an agreed fraction of the time. To put the space station together, each nation is responsible for getting its own pieces into orbit. ESA and Japan intend to use their own systems, the Ariane and H-2 rockets, and would have to compete with everyone else if they wanted to buy flights on the shuttle.

According to Dr Heinz Riesenhuber, West German Minister for Research and Technology and also chairman of the ESA ministerial council, the disagreements over the nature of research to be done on the space station were settled by first observing that it would be impossible to formulate definitions of civil and military research that all parties could agree on. The essence of the consensus reached is that each nation can do in its own facilities what it wishes, and can deny use of its facilities to other nations only if sensitive research is proposed. Riesenhuber says this agreement does not pretend to provide a foolproof way of solving any disputes that might arise, but establishes an etiquette so that amicable partners can avoid them. A good contract, he said, is one which once signed "need never be read again". Only nine of the thirteen ESA members are taking part in the space station programme, but according to Reimar Lüst, ESA's director-general, the non-participation of the others was not a sign of dissatisfaction with the terms, because all thirteen had to approve ESA's role as participating agency for the nine.

David Lindley

■ Shinichiro Ogura, space station chief at the Science and Technology agency, says that Japanese requirements that the space station should be a purely civil venture have also been met. Beyond its interest in microgravity research and Earth observation programmes, Japan sees its participation as a way to learn about and contribute to space technology. Japan has done little in space so far, but there is talk of an ambitious programme stretching into the next century, including a Japanese shuttle (*Nature* 327, 645; 1987). Although in the present climate the prospect of Japan gaining access to US expertise might have been expected to cause friction, Margaret Finarelli, policy director in the Office of Space Station at NASA, says this was not an issue in negotiations. The different modules will be plugged together in a 'clean' way, she says, and the construction of the various modules will be entirely in the hands of their owners. In hardware alone, the space station will cost the United States \$16 thousand million, with ESA, Japan and Canada putting in four, two and one thousand million dollars respectively. Some 20 shuttle launches will be needed, at perhaps \$100 million each, for the US sections alone. Both Finarelli and Riesenhuber expressed the hope that, with an international agreement to honour, the US Congress would be under some pressure to keep the money flowing from one yearly budget to the next.

David Lindley & David Swinbanks

US role alleged in cover-up of researchers guilty of war crimes

Cologne

NEW evidence that the United States helped to cover up crimes committed during the Second World War by Japanese and German physicians was presented last week at a conference here entitled "Medical Science without Compassion, Past and Present".

Historians, physicians and genetecists spoke at the conference, which was organized by geneticist Benno Müller-Hill. Most talks concerned Nazi medical crimes — the participation of doctors and especially psychiatrists; the continuity of the German sterilization and euthanasia programmes of the 1930s with the murder of Jews and gypsies; and the lack of contemporary or even subsequent condemnation by the medical and scientific community. Müller-Hill said that he organized the conference because "the attack on Nazi science in West Germany had been left to what I'd call anti-science groups" and "I'd like to take this topic away [from them]." This conference was supported by the Deutsche Forschungsgemeinschaft, a research funding organization.

The alleged use of prisoners in biological warfare experiments in Manchuria by Japanese physician Shiro Ishii was described by Sheldon Harris of California State University at Northridge. Harris claimed that Ishii moved to the United States in 1948 and lectured at the US biological weapons research station at Fort Detrick, Maryland. No Japanese physician was ever prosecuted for war crimes.

"At least 3,000 prisoners" died in Ishii's experiments at the Ping Fan camp in Manchuria between 1937 and 1945, said Harris. Ping Fan was established under the pretence of being a lumber mill, and the prisoners were allegedly known to their captors as "logs."

Ishii was said to have studied the immunological response against pathogens ranging from typhoid and dysentery to anthrax. He also developed vaccines against diseases caused by these and other agents. The Ping Fan facility produced at least 20 million doses of vaccine annually.

Harris cited official denials that human subjects were used in Japanese biological warfare experiments. But Harris said that a US intelligence report from 1947 noted that "experiments on humans were known to and described by three Japanese and confirmed tacitly by Ishii".

A 1947 official report submitted by a US Navy commander to the State War Navy Coordinating Subcommittee for the Far East stated that "at least 12 bacteriological warfare weapons field trials were conducted against Chinese military and civilian populations". Harris claimed that US

prisoners were also used for the experiments, although the report denied this.

Humans were used for both laboratory testing of the immune response, according to the report, as well as for field trials. According to Harris, Ishii's capture by US occupation forces in Japan in 1946 was considered "a great coup for the advancement of US biological warfare research". Negotiations with Ishii lasted throughout 1947, said Harris, with Ishii offering as bargaining chips "tantalizing disclosures about human experimentation".

US negotiators informed higher authorities all the way up to the Joint Chiefs of Staff, said Harris, who then authorized each succeeding round of negotiations. US leaders were allegedly concerned as early as 1947 that the US government might be "seriously embarrassed", according to the minutes of the State War Navy Coordinating Committee, if it came out that these negotiations were carried out with Japanese physicians at the same time as German doctors were being prosecuted at Nuremberg for experimenting on humans. But interest in Ishii's experiments, said Harris, overrode all moral and political concerns.

West German physician Christian Pross revealed new evidence on the dual role played by one US investigator, the Vienna-born Boston neurologist Leo Alexander, in preparing war crimes cases while looking for "valuable results" that could be used by US medical science.

Pross contends that Alexander's reports reflect a mixture of ethical condemnation of the Nazi scientists and admiration of their "objective and accurate observation and interpretation". Alexander, he said, had worked in Germany until the Nazi takeover forced him to flee in 1933.

Pross sees evidence for US government manipulation in the change of heart of Andrew Ivy, a physiology professor at the University of Illinois, Chicago, in his Nuremberg testimony at the so-called "doctors' trial" of 1946–47. Ivy had filed a report condemning two of the German physicians on trial as accessories to the notorious Sigmund Rascher in his hypothermia and high-altitude experiments on human subjects at Dachau. The physicians, Siegfried Ruff and Hans-Wolfgang Romberg, claimed that they had not done any experiments in which subjects died.

On the witness stand, Ivy made very different statements. He defended the legitimacy of using human subjects for wartime research. Ruff and Romberg were acquitted and Ruff went on to become director of the Aeromedical Institute of the Bundeswehr, West Germany's army.

Steven Dickman

SDI chief quits as funds are cut

Boston

LT-GENERAL James Abrahamson, director of the Strategic Defense Initiative (SDI), has resigned his post with effect from January 1989, the Pentagon unexpectedly announced last week. The announcement of Abrahamson's resignation came the day before Congress approved a military spending bill that will halt the growth of funds for the programme for the first time in its five-year history.

Abrahamson's departure, which will coincide with that of the Reagan administration, is expected by many analysts to mark the end of an era for the controversial anti-missile defence system.

The timing of Abrahamson's decision to leave seems clearly tied to prospects that a new administration will probably scale down the project's budget, and the attention given to it. In his letter of resignation, Abrahamson stated that "a new administration will undoubtedly have different ideas or approaches" to SDI and that "new leadership" would best represent the "new policy and direction".

While the Congress has agreed to allot \$4.1 thousand million to the SDI programme for fiscal year 1989 (which began on 1 October), the stagnation of the budget comes after many years of sharp increases. It seems generally agreed that in either a Bush or a Dukakis administration, this figure will represent a "high-water mark" for the programme. Arms-control expert Ashton Carter, acting director of the Center for Science and International Affairs at Harvard University, predicts that, without President Reagan's strong advocacy, it is likely that SDI will return to the status of "just another R&D project" at the Pentagon.

Vice-President Bush, in his campaign for the presidency, has affirmed his support for the SDI programme, but Carter says that pressures to restrain the federal budget may be strong enough to force reductions under any administration. Governor Dukakis has stated that, if elected, his administration would cut the project's funds by as much as \$3,000 million.

During his tenure, Abrahamson has opposed vigorously any proposals to limit the programme's scope or delay its ambitious plan to begin testing by the 1990s.

Abrahamson, 55, has been head of the research programme since 1984, when the position was elevated to a special new post in the Pentagon. On his departure, he will retire from active military duty. The Pentagon has nominated as his successor Lt-General George Monahan Jr, now the Principal Deputy Assistant of Acquisition for the Air Force. His nomination must be confirmed by Congress.

Seth Shulman

New rules for US research grants prove a popular choice

Big Pine Key, Florida

SOME of the layers of bureaucracy separating researchers and the agencies which fund their work will be peeled away under a nationwide grant administration programme due to begin this month. The Federal Demonstration Project will bring together eight government agencies and over 35 universities to try out new streamlined procedures for administering federal grants.

The national project is an extension of a successful smaller test programme begun

over two years ago and involving several universities in Florida. The experiment linked the National Institutes of Health (NIH), the National Science Foundation (NSF), the Departments of Energy (DoE) and Agriculture and the Office of Naval Research with the Florida State University System and the University of Miami.

The objective was to tell whether simplifying the grant reporting process could enhance research productivity without compromising the accountability of the grant recipients. In the test project, each of the agencies, as now, assessed the scientific quality and financial responsibility of grant programmes before and after they were awarded. But while grants were current, many of the management issues on which agency approval would normally have been required was delegated to the researchers themselves.

In particular, researchers were permitted to start spending their funds as much as 90 days before the formal starting date, and to carry forward unspent money into subsequent periods. They were also allowed to spend funds on foreign travel and permanent equipment, to make line-item changes in their budgets and to extend their grants for up to twelve months without agency approval.

Most federal agencies, including some not directly participating in the project, are now working towards using the new procedures. NASA has already implemented many of the reforms. Now the

Veterans Administration, the Department of Education and the Nuclear Regulatory Commission are considering using all or part of the procedures soon. NIH, NSF and DoE also plan to begin using them to administer nearly all their grants, not just those received by participating institutions.

Even so, the participating institutions are not claiming that the new arrangements are a way of saving money. Don Phillips, head of the National Academy of Sciences' Government-University-Industry Research Roundtable (GUIRR), which coordinated the experiment, says the two most important results of the Florida project were that it showed that federal agencies could work together to reduce red tape, and that doing so would improve researchers' attitudes towards the sponsored research system and give them more time to do research.

The difficulty with the current grant system, members of the Roundtable say, is that federal research support has acquired administrative, managerial and financial procedures which hinder research by treating it as a procurement system rather than as a long-term investment. The approach forces research programmes to be compartmentalized into "arbitrary project units", leading to "transaction-by-transaction accountability" and lack of flexibility. Treating research as an investment instead produces less friction and enhances productivity.

For the federal project, the Environmental Protection Agency, the research arms of the Army and Air Force and 26 more universities will be added to those who participated in the Florida project.

Martha Cleveland

Olympic dope-test net misses Poles

Warsaw

THE Polish government last year invested \$900,000 of its scanty hard currency reserves in establishing a state-of-the-art centre for testing athletes for drugs. Yet according to the Communist Party daily, *Trybuna Ludu*, three of the Polish squad at Seoul had no right to be there. Track-and-field athletes Jolanta Janota, Ewa Pisiewicz and Agnieszka Siwek, said *Trybuna Ludu*, had refused anti-doping tests during the Polish national games in August, and according to the Polish rules "refusal to submit to an anti-doping test is equivalent to testing positive". Poland's chief track-and-field coach, Honorat Wisniewski, issued a formal protest, saying that he and his fellow coaches "would not take responsibility for the effects, whether in or outside of sport, of sending these three competitors to the Seoul Olympic Games". The members of the Polish "Anti-Doping Commission", *Trybuna Ludu* suggested, had simply "hidden their heads in the sand" in allowing the women to go.

In Seoul none of the women finished in the first four places for which testing is mandatory, nor were they selected for random testing. Unlike Calgary, therefore, Seoul posed no threat to Poland's national honour. But the incident throws some doubt on the Polish authorities attitude to their own 'Anti-Doping Commission', and the *Trybuna Ludu* comment is less than fair to the two scientists from the anti-doping laboratory who are also members of the commission. For the decision to send the three women to Seoul was taken when both scientists were absent from Warsaw — one on vacation, and one carrying out routine anti-doping tests elsewhere in Poland. No attempt, apparently, was made either to recall them or to postpone the meeting of the commission for a day or two until at least one of the scientists could be present.

Vera Rich

US places further regulations on pesticide manufacturers

Washington

AFTER two years of wrangling, Congress last week passed a bill authorizing the re-examination of over 600 previously approved pesticides, to allow a more complete assessment of their effects on the environment and public health. The bill amends the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) — the major piece of legislation governing pesticides in the United States — to require manufacturers to submit new data on pesticides approved before the end of 1984.

The bill represents a compromise between environmentalists, who had hoped for stiffer laws governing pesticides, and the chemical industry. Pesticide manufacturers will be required to pay for the re-testing process and to subsidize part of the \$42 million per year the government is estimated to spend on evaluating the new data. But it does not contain provisions to

protect groundwater supplies from contamination, to identify violators and force them to pay for clean-up procedures, or to clamp down on exports of pesticides unapproved in the United States, items the environmentalists would like to have seen included.

One of the most controversial points of the bill concerned the alteration of the 'indemnification' clause of the old law, under which the Environmental Protection Agency (EPA) was obliged to reimburse a manufacturer for stockpiles of approved pesticides which it later banned. The obligation has slowed down the removal of dangerous pesticides from the market in the past because the government could not afford the payments. The current bill takes away the indemnification requirement, and allows the EPA instead to consider payments to manufacturers on a case-by-case basis.

Carol Ezzell

Call for total ban of production and use of chlorofluorocarbons

Washington

THE administrator of the US Environmental Protection Agency (EPA), Lee Thomas, called last week for the phased total elimination of chlorofluorocarbons (CFCs), used as refrigerants and solvents and in the manufacture of foam packaging, and which are destroying the stratospheric ozone layer. The agency had previously supported the 50 per cent reduction plan set out in the Montreal Protocol negotiated last year between 45 countries, and now ratified by eight. Thomas is still urging all nations to ratify the protocol, but says the "global environmental community must go even further to eliminate these chemicals".

Thomas' announcement, favouring a complete halt to the use and production of CFCs, was based on data presented in a report, commissioned by the EPA, that modelled the environmental consequences of several reduction alternatives. The report stated that even if every country in the world were to participate in the Montreal Protocol, the level of chlorine being released into the atmosphere would nearly triple by the year 2075. Because CFCs break down slowly in the

atmosphere, the report says, their total elimination would still cause chlorine levels to rise for the next six to eight years.

The report also says that methyl chloroform and bromine should be eliminated in order to protect the ozone layer. Methyl chloroform, an industrial solvent, is expected to be the major source of chlorine under the Montreal Protocol, because it will be used increasingly as an alternative to CFCs. Bromine, another ozone-depleting halogen, is now used in fire extinguishers. A total ban on CFCs, bromine and methyl chloroform is projected to keep atmospheric levels of halogens stable for the next 100 years.

The Alliance for Responsible CFC Policy, an international group of producers and industrial users of CFCs, also announced its support of a total CFC ban last week. Du Pont, a member of the alliance and one of the major producers of CFC refrigerants, unveiled plans to begin selling a series of alternatives after 1990. Du Pont said this week it is building a plant for the production of HFC-134a — a hydrofluorocarbon not containing chlorine — to replace CFC-12, the most widely used refrigerant. Carol Ezzell

FDA urged to review wonder "smokeless" cigarette for safety

Boston

SHOULD a new cigarette described by its manufacturer as "a major technological breakthrough" be subjected to scrutiny by the Food and Drug Administration (FDA)? The new product is due to go on sale in three test markets this week, but a coalition of health associations, including the American Medical Association and the American Cancer Society, have filed a legal petition with FDA, asking the agency to review the product's safety as if it were any other drug.

FDA has traditionally stood aside from the assessment of tobacco products unless manufacturers have made explicit health claims for their product. Regulation so far has been limited to smoking accessories, such as filters meant to reduce intake of nicotine, as well as other 'nicotine delivery products', such as chewing-gum.

R. J. Reynolds tobacco company, which is offering the new cigarettes, called 'Premier', has been careful not to make overt health claims about its product, which is to be advertised as offering "cleaner enjoyment". But there is little doubt that the new product marks a radical departure from previous cigarettes. 'Premiers' have been called "smokeless"

cigarettes because they are the first not actually to burn tobacco; instead, tobacco is merely heated with the help of an elaborate internal carbon heating element.

In reality, the high-tech cigarettes are not strictly smokeless: they do provide nicotine-laden vapour which the smoker inhales, but they do not give off ambient smoke at the distal end. This feature is a clear effort to meet public concern about the risks of passive smoking as well as to stem the tobacco industry's declining sales in the United States, which have fallen nearly 2 per cent a year since 1982.

The petitioning health associations assert that the new cigarettes should receive FDA review in part because Reynolds, in its patent application for the new cigarettes, lists "pharmacologically or physiologically active agents" (including ephedrine and metaproteranal).

Activists in at least one of the test markets, Phoenix, Arizona, say they are considering filing for an injunction against the product until FDA rules on its safety. Says Dr James H. Sammons of the American Medical Association, "We are simply unwilling to accept on faith the new product of an industry that still denies that smoking is unhealthy". Seth Shulman

Du Pont had nuclear reactor problems

Washington

DETAILS were made public last week of as many as 30 "significant" nuclear reactor incidents that have occurred since 1957 at the Savannah River Plant in South Carolina, a complex of reactors where fuel for nuclear weapons is produced. The most serious incident occurred in 1960 when an attempt to restart one reactor very nearly led to an uncontrolled chain reaction.

The incidents were revealed last week during a joint hearing of the Senate Committee on Government Affairs and the House of Representatives Government Operations Subcommittee on the Environment.

The Savannah River Plant is operated by E. I. du Pont de Nemours & Company under contract to the US Department of Energy (DoE). Du Pont denies charges that it had made any attempt to hide the nature of these incidents from government officials. A DoE spokesman said that the agency is still reviewing its records to determine whether Du Pont's reports were accurate and complete.

Other incidents revealed in a 1985 memo from a Du Pont employee include the unexpected power surges (1964); fuel rod melting (1970); and loss of scram capability for up to 40 days (1964).

The hearing was called to investigate the adequacy of DoE's oversight of safety concerns at its reactors, and specifically problems related to the restart of the P reactor at Savannah River. Du Pont has elected not to renew its contract to operate the Savannah River Plant when it expires at the end of March 1989. Joseph Palca

No fallout likely from Cosmos 1900

Washington

THE nuclear reactor on board the falling Soviet satellite Cosmos 1900 (*Nature* 335, 199; 1988) has been propelled into a high orbit by an automatic safety mechanism, and no longer threatens to fall to Earth. The slightly elliptical 700-kilometre orbit is well beyond the atmosphere, and will be stable for centuries, by which time the radioactivity will have largely subsided. According to Major Alex Mondragon of the US Air Force Space Command in Cheyenne Mountain, Colorado, the ejection of the reactor also sent two fragments of Cosmos 1900 into the atmosphere above the north-west and south-east coasts of Africa, but no sightings of the debris were reported, suggesting that they were small enough to burn up as they descended. Mondragon said that Space Command is certain the nuclear reactor is aboard the piece sent into high orbit, but "can't explain" why. David Lindley

UK government's plans to split teaching and research

London

FUNDS for teaching and research in universities should be allocated separately, says the British government. But the universities are not so sure. And the issue refuels the debate over the link between excellence in teaching and in research which looks set to be top of the agenda in future discussions between the academic community and the government.

Mr Kenneth Baker, the Secretary of State for Education and Science, delivered the government's message to the universities Committee of Vice-Chancellors and Principals (CVCP) at their annual meeting last week. After the meeting, vice-chancellor of the University of Bristol, Sir John Kingman, called Baker's message an "extremely sinister development". "Most vice-chancellors are uneasy about it. It could destroy the quality of universities in Britain."

The crucial decisions on funding will be made in the course of negotiations between the universities, the new Universities Funding Council and the government, next year. For their part, universities are willing to provide services which reflect the funds allocated, and for the quality of the service to be monitored. What they will not tolerate, said Kingman, is interference in the way universities achieve that quality.

But Baker said that separation of funding would raise standards by allowing some academics to concentrate on research and others to concentrate on teaching and administration. He said more emphasis should be placed on teaching, and that "universities should assign to teaching the prestige they have traditionally assigned to research".

Later in the week, the CVCP said it is setting up a new academic standards unit which will review procedures for monitoring and improving academic standards and offer advice to individual institutions. The CVCP also plans to undertake a study of teaching costs and prices similar to that undertaken recently for research.

Baker said he recognized that various practical questions would have to be answered before a scheme for the separation of teaching and research could be fully implemented. But there are strong feelings within the universities that such a separation would be destructive. There is also widespread opposition to a rigid three-tier system of universities and at the meeting, Baker said the government will not enforce a three-tier division of universities according to the labels: type R (with substantial research across all fields); type X (with substantial research in particular fields); and type T (with just the research

necessary to support teaching but without advanced facilities). But it is clearly the government's intention to distribute resources for teaching uniformly and allocate greatly differing funds for research, says Kingman. And Peter Thorpe, secretary of the Advisory Board for the Research Councils which recommended the RTX system, said that the rejection of rigid labelling of the universities does not mean such a system will not evolve. Sir Walter Bodmer, president of the British Association for the Advancement of Science, says that divisions already exist, but stresses that they should not become too rigid.

Baker plans more upheaval within universities which by the end of the century will result in "a distinctly different

regime from the one we have now". And the question of whether universities will retain their autonomy did not disappear after the passage through parliament of the 1988 Education Reform Act. In this respect, the drawing up of the arrangements between the universities, the new Universities Funding Council and the government will be crucial. Baker took care to put the "sound and fury" of the last few months behind them and reassured the universities that they "have nothing to fear from the government. We want you with us, not against us."

Baker also said that the universities should increase their efforts to obtain funding from private sources, business and alumni. The CVCP responded with a request for alteration of tax regulations to encourage such donations. The government also wants more emphasis on the consumer — universities should be better tuned to the desires of the students, said Baker.

Christine McGourty

Margaret Thatcher's U-turn on support of basic research?

London

THE government must take full responsibility for supporting basic science in Britain; it cannot rely on the private sector to do so. That is usually the cry of the academic community in the face of dwindling resources for scientific research. But last week it came from the Prime Minister, Mrs Margaret Thatcher, in a speech to the Royal Society, which seems to mark a change in government thinking.

The Times

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Mrs Thatcher with Sir George Porter, president of the Royal Society, last week.

This government is not renowned for appreciating the importance of basic research. It has continually stressed exploitability and encouraged industry to play a greater role in supporting basic research. But that is changing. Sir Walter Bodmer, president of the British Association for the Advancement of Science, says that a change of attitude can be sensed within the cabinet, particularly in the views of the chief scientific adviser John Fairclough.

And Thatcher's speech last week indicated a significant shift in government thinking, says Denis Noble, president of the pressure group Save British Science.

But the scientific community is not rejoicing yet: there were no specific declarations of increased funding. "We want to see the colour of their money", says Bodmer. Noble agrees that no one will know the government's true intentions until the science budget is set later this year.

The speech was particularly interesting for those concerned about the environment. The greenhouse effect, the ozone layer and acid rain were all subject to the concern of the prime minister last week. Money spent on safeguarding the environment "is money well and necessarily spent", she said, "because the health of our economy and the health of our environment are totally dependent upon each other". "Stable prosperity can be achieved throughout the world provided the environment is nurtured and safeguarded. Protecting this balance of nature is therefore one of the great challenges of the late twentieth century."

Some might be sceptical of the motivation for such concern, coming during a period when the resources of the Natural Environment Research Council (NERC) have been drastically squeezed, and when Britain is accused of dragging its feet on environmental legislation in Europe. But the chairman of the NERC, Sir Hugh Fish is encouraged by her concern and now expects to see more funding available for research in these areas. His researchers take a less generous view. One called it "all talk", inspired by political motives. But if more resources are forthcoming few will question whether the concern is genuine or not.

Christine McGourty

Placebos prompt new protocols for AIDS drug tests

Berkeley

THE availability in the United States of unproven AIDS drugs, while wreaking havoc on controlled drug trials, is also impelling researchers to take a hard look at the design of drug trials and the ethics of those procedures.

People with AIDS, poised as they are on a knife-edge between self-preservation and altruism, often make decisions on whether to comply with the rules of a trial based on their sense of its fairness. Concern that patients may not fully comply with the rules of trials in which they are enrolled has now stimulated a search for mutually acceptable protocols.

Placebo controls are the most highly charged issue, and possibly the most widely misunderstood. Some researchers believe that people are unaware that, once a trial drug is found to be effective for the treatment of a fatal disease, it replaces the placebo as the standard against which potential treatments are tested.

But in the absence of a proven treatment, trial designers face an ethical paradox, according to Yale medical ethicist Robert J. Levine. While testing a drug in patients is warranted only if there is reason to expect it will work, it is considered unethical to design a study in which some patients are certain to fare worse than others. The use of placebo controls becomes questionable if there is strong preliminary evidence that a drug is effective, and battles often rage over the quality and implications of this evidence.

One such controversy surrounds Protocol 019, a trial of AZT in asymptomatic HIV-seropositive individuals being carried out at several centres. The objective is to tell whether treatment with AZT will affect the progression of those infected with HIV progressing to later stages of the disease.

The study is placebo-controlled, says principal investigator Paul Volberding, of San Francisco General Hospital, because there is no proven treatment for asymptomatic HIV infection. But Martin Delaney, of San Francisco's Project Inform, an AIDS education group, argues that, because of ample anecdotal evidence of the effectiveness of AZT, the study's placebo arm is essentially asking 1,000 patients to "fall on their swords".

In order to protect those receiving placebos, Delaney therefore argues that the clinical endpoints of the study should be more sensitively defined, and that clinical measures of progression to AIDS or AIDS-related complex (ARC) should be replaced by criteria such as the reduction of a blood parameter such as T4 cell count below a predetermined level.

Volberding defends the trial's design, claiming blood parameters are not reliable indicators of AZT's effectiveness. He notes that Protocol 019 may be the last placebo-controlled trial for AIDS drugs, if it proves AZT to be effective at all stages of HIV infection.

The opposition to placebo controls has driven some researchers to "telescope" drug trials by including a placebo arm in phase I safety studies, so that any early signs of a drug's efficacy can be balanced against the effects of a control. San Francisco AIDS researcher Donald Abrams is conducting such a trial of lentinin, a potential booster of the immune system extracted from shitake mushrooms. If the drug shows promise, then at least some placebo-controlled data will exist, says Abrams, in case there is resistance to a later protocol using placebo.

Mistrust of the design of trials can stifle a patient's altruistic inclinations and spur him to exploit trials in which he is enrolled for personal ends, says New York patient-advocate Tom Hannan. He tells of participants in early AZT trials who concealed their use of other drugs, or had their pills analysed at independent laboratories and left the study if they had been assigned to the placebo group. Volberding believes that stories such as these have been exaggerated.

The spectre of non-compliance casts a shadow of doubt on nearly all AIDS drug trials so far. The Food and Drug Administration's (FDA's) recent relaxation of

restrictions on the import of unapproved drugs has made controlled testing of dextran sulfate impossible in the United States, according to Donald Abrams, who found that subjects in his phase-I toxicity trial were supplementing their doses with drug obtained elsewhere.

Some believe compliance can be improved through community-based trials, in which participants are supervised by their primary-care physician. Patients are more likely to follow the rules if a trusted personal physician has helped to design them, says Tom Hannan, who heads New York City's Community Research Initiative, one of several consortia using this approach.

But some researchers question whether the physicians have the experience necessary to execute sound and unbiased trials. Jack Killen, deputy director of the AIDS programme at the National Institute of Allergy and Infectious Diseases (NIAID) said the institute plans to fund more community-based trials, but not at the cost of scientific soundness.

Perhaps the most obvious, but also most problematic, way to deal with self-medicating patients is simply to monitor their medication and clinical progress. Long advocated by AIDS patients, this idea is taking hold in the research community.

While NIAID has shown interest in these monitoring studies, Killen says they would be most useful in eliminating drugs that show little promise. But what if a drug were to show promise, and the supporting evidence, while not rigorous enough to pass FDA approval, complicated the ethics of setting up a controlled trial? Says Killen: "I don't know the answer to that question."

Marcia Barinaga

Medvedev promoted in Soviet shake-up

London

VADIM Medvedev, a former head of the Science and Education Department of the Central Committee of the Communist Party of the Soviet Union, was last week elected to full membership of the Politburo, the supreme decision-making body of the Soviet Union.

He has, moreover, been appointed chairman of the newly created "Commission for Ideological affairs", thus effectively replacing as chief party ideologue the traditionalist Egor Ligachev, who for three years has been Mr Mikhail Gorbachev's main rival for the leadership. Now Gorbachev holds the dual position of President and General Secretary of the Party and Ligachev has been put in overall charge of Soviet agriculture, a position from which it is virtually impossible to succeed.

Medvedev, who is 59 years old, is very much a Gorbachev man. An economist by training, he held a succession of academic posts, rising to be Rector of the Party

Academy of Social Sciences (1978-83) and then head of the Central Committee's Science and Education Department. But these, and other posts (such as the deputy chairmanship of the Propaganda Department of the Central Committee) which he held simultaneously with his academic appointments, left him at best on the periphery of political life. Under Gorbachev, however, he was moved to the important "career" post of Central Committee secretary in charge of liaison with ruling communist parties.

Having thus been the party specialist on science and education during the final "years of stagnation" and being himself a corresponding member of the Soviet Academy of Sciences, Medvedev is undoubtedly well briefed on the hard core of "conservatism" within the academy. Any pronouncement he may make in the future on the need to reform the scientific establishment will come, therefore, from a position of strength.

Vera Rich

Observation satellite planned for the 1990s

Tokyo

JAPAN'S National Space Development Agency (NASDA) plans to launch a large polar-orbiting Earth observation satellite in 1993 to monitor the Earth's ozone layer. If the plans come to fruition, the satellite will join two other polar-orbiting platforms, one part of the US space station project (see page 480) and the other built by the European Space Agency.

The Advanced Earth Observing Satellite (ADEOS) will be placed in orbit by an H-2 rocket, and will carry an ocean colour and temperature sensor, a visible and near-infrared radiometer to observe land and coastal areas and an ozone sensor. Besides its global monitoring tasks, ADEOS will also participate in intersatellite communication experiments with the Engineering Test Satellite-VI, a geostationary communications satellite scheduled for launch in 1992. NASDA hopes to launch an experimental data relay and tracking satellite in 1994 to enable real-time communications with ADEOS when it is out of the range of ground tracking stations.

Most of the satellite will be built in Japan, but NASDA has invited international scientific participation in the development of the ozone sensor. Eight applications have been received from the United States, Italy, France and Australia. According to a NASDA official, the strongest proposal is from the US National Aeronautics and Space Administration (NASA), which wants to place a Total Ozone Measuring Sensor (TOMS) on ADEOS.

A TOMS sensor aboard NASA's Nimbus 7 satellite confirmed the existence of the hole in the ozone layer over the Antarctic two years ago after measurements from ground stations first indicated ozone loss, and this led to an international agreement to limit the use of chlorofluorocarbons. In August this year, NASA and the US Environmental Protection Agency met Toshio Horiuchi, the director-general of Japan's Environment Agency, to discuss the possibility of carrying a TOMS sensor on ADEOS. But Shinichi Ogura of the Science and Technology Agency, to which NASDA is affiliated, says a final decision on the sensor has not yet been made.

NASDA has applied for ¥520 million (US\$4 million) for research and development of ADEOS during fiscal year 1989. A NASDA official estimates it will cost roughly US\$400 million to build the 3-ton satellite.

David Swinbanks

French government approves abortion pill for commercial use

Paris

A DRUG which can be used to terminate an unwanted pregnancy without the need for hospitalization has now been approved for commercial distribution in France. So far, China is the only other country to have given approval to this drug, known as RU-486. But fears of an over-the-counter abortion pill (see *Nature* 325, 185; 1987) look to be unfounded. The French Ministry of Health approval, which was given last week, limits distribution of RU-486 to selected clinics where strict precautions and follow-up should be assured.

RU-486, or mifepristone, was discovered in 1982 by a French researcher, Etienne Beaulieu, and is marketed by Roussel-Uclaf laboratories under the brand name Mifegyne. The drug acts by 'squating' cells in the uterus which normally act as receptor sites for the hormone progesterone. Without progesterone the endometrial lining of the uterus breaks down, causing bleeding and stimulating the release of prostaglandins. The resulting muscle contractions usually lead to the embryo being expelled. It is this action, which prevents pregnancy being established that led Beaulieu to call the drug a 'contragestive' and not an abortifacient.

The French government approval contains several precautions to limit abuse as well as to enhance the effectiveness of RU-486. First, the drug may be administered only in association with prostaglandins. Clinical trials in France and abroad showed that a 600-mg dose of RU-486 alone led to successful expulsion of the embryo in about 80 per cent of cases but that the success rate is greatly improved if the dose is accompanied by prostaglandins, which aid contraction of the uterus.

Second, the drug is to be given only during the first 49 days of pregnancy. After this time the woman's body produces too much progesterone for RU-486 to be effective. Since 1974, abortion, usually by vacuum aspiration of the foetus, has been legally available in France in specialized family planning clinics, up to the twelfth week of pregnancy. Existing law requires an 8-day 'thinking period' once a woman has asked for her pregnancy to be terminated. This effectively means that a woman wishing to be given RU-486 must be able to satisfy doctors that she is less than four weeks pregnant. Currently, only about half of the 177,000 women seeking abortions each year would meet this criterion.

After the 8-day 'thinking period' a 600-mg dose (3 tablets) of RU-486 is taken orally in the presence of a doctor. Two days later, when bleeding should have

begun, the doctor administers prostaglandins by intramuscular injection or with a vaginal suppository. The patient is then asked to return for an echograph to ensure that the embryo has been expelled. In the event that the drug has failed to terminate the pregnancy — a less than 3 per cent chance so long as RU-486 is associated with prostaglandins — arrangements are then made for a conventional abortion.

According to Professor Roger Henrion, head of the maternity department at the Port-Royal hospital in Paris, who chaired the Ministry of Health commission, a woman who changed her mind at this stage and decided to have her baby would stand an as-yet unknown risk that the infant would be deformed. Studies in the rabbit have shown there is a risk that the non-expelled fetus will not develop normally and there has already been one case in France of a deformed baby being born to a woman who received RU-486 during pregnancy.

Henrion admits that a black market for RU-486 could emerge but hopes that the media will help emphasize that RU-486 "is not the painless trouble-free, do-it-yourself abortion pill which some women have been led to believe. If what is stipulated by the government authorization is respected, I can only see an improvement in the choices available to women. But if this product finds its way out of these centres, or if doctors are not careful, very quickly there could be very serious risks."

According to Henrion, 18 to 20 per cent of women taking RU-486 continue to bleed for longer than 12 days. There are also risks that the embryo will not be expelled, or that the placenta will be retained, leading to infection. Henrion also fears the return of rhesus immunization, whereby a rhesus positive fetus of a rhesus negative mother induces production of antibodies which could endanger future pregnancies. Simple precautions are taken within the authorized clinics to overcome this risk.

French researchers are now looking at the effectiveness of RU-486 during the second cycle of ovulation as a means of birth control — its main function in China. The World Health Organisation has also asked that Roussel-Uclaf make the drug available at reduced cost to developing nations. In the meantime, in several other countries, including the Netherlands, Britain and Spain, application has been made to legalize RU-486 for abortion.

Peter Coles

● See pages 492 and 543 of this issue for news of an effective contraceptive based on a sperm protein.

Rocky start for new biology building on Californian campus

Berkeley

THE problems plaguing the new Life Sciences Building Addition (LSA) on the Berkeley campus of the University of California are either unusually serious consequences of poor design and construction or just the normal fine-tuning that a new building must experience.

In a classic case of where-you-stand-depends-on-where-you-sit, those happily settled in the new building, with money for necessary retrofits, say the problems have been exaggerated. But those who have been forced to wait more than a year to move in tell horror stories of the causes that have delayed their occupancy.

The LSA is part of a large building-programme now under way at Berkeley. A second new biology building is nearing completion, and work will begin shortly on a complete remodelling of the venerable Life Sciences Building. All this coincides with a reorganization of graduate biology education at Berkeley (see

usable and drains in the animal care facilities had obviously been sited at the high points of sloping floors.

But the worst problem was noise. John Miller, a neurobiologist working on cerci, a sensory organ of crickets, says that even with damping, the vibration caused by the air rushing through the ventilation system will force him to abandon his research model unless extensive changes are made. Even for those whose research is not hindered by it, the noise has made some reluctant to move into the building.

But researchers who have moved in say the building is working out just fine. Biochemistry professor Gerald Rubin acknowledges that there have been some problems, but says that minor adjustments have cleared them up. Louise Taylor, special assistant to the vice-

chancellor who is responsible for the LSA, says the building's problems were merely routine, and that people have been clamouring to use the building.

Taylor says occupancy is now 42 per cent, and growing. She maintains that the "air exchange problem is not an issue", and that noise problems that seem severe in an empty laboratory disappear when people and equipment are moved in. She adds that Stent, one of the first to complain about the noise, may have had an exaggerated perception of the problem because he wears a hearing aid.

Although the new building has inflamed passions among the Berkeley biology faculty, most agree that the problems are on their way to being solved. An engineer hired to oversee retrofit efforts earlier this summer has evidently found cost-effective ways of solving many of the problems, and even the building's severest critics feel the worst is over. Says Miller, "Ultimately it's going to be a great place to be — when it's fixed."

Joseph Palca

British charity deal with Japan on anti-cancer drug technology

Tokyo

THE flow of British drug technology to Japan may increase substantially with the signing last week of an agreement between Sumitomo Corporation and the commercial arm of Cancer Research Campaign (CRC), a charity which funds much of Britain's cancer research.

Under the agreement with CRC Technology Ltd, the wholly owned company of the campaign, Sumitomo will act as sole agent in Japan to license inventions and drugs arising out of CRC-funded research. Sumitomo made a similar arrangement with Britain's Medical Research Council in July (*Nature* 334, 3; 1988); the two agreements offer Japanese corporations the opportunity to develop the inventions of a large sector of Britain's medical research community. Hitherto, Japanese pharmaceutical and chemical companies have relied heavily on the United States as a source of new inventions in the medical field.

CRC, with an annual budget of about £30 million derived from donations and legacies, supports four major cancer research centres in Britain (the Institute for Cancer Research, the Beaston Institute of Cancer Research, the Gray Laboratory and the Paterson Institute for Cancer Research), provides grants to over 60 university medical departments, and also supports two centres for clinical trials of new anti-cancer drugs.

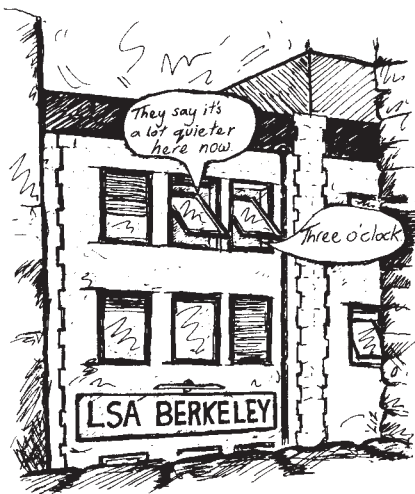
CRC Technology Ltd was established in 1982 to transfer technology developed through CRC-funded research to the private sector. Formerly called Carlton

Medical Products Ltd, the company was revamped and renamed last year in line with government policy to strengthen links between industry and universities.

CRC plays a leading role in developing and testing new anti-cancer drugs in Europe and North America. But, according to Sue Foden, Managing Director of CRC Technology, no CRC drugs or technology have been developed or tested in Japan. She foresees many opportunities here, particularly for drugs to treat cancers, such as stomach cancer, that are common in Britain and Japan. And she hopes that the agreement will lead to a "large flow of money" back to Britain to support further campaign research. Foden says it was recently agreed that the campaign will hold the patent rights to campaign-funded research but profits from licensing will be shared equally with the British research organizations where the inventions are made.

Takao Hirose of the New Drug and Biochemical Development Department at Sumitomo says "many" Japanese pharmaceutical and chemical companies are interested in developing British anti-cancer drugs. Japan's anti-cancer drug market is one of the world's largest but at present it is dominated by drugs of questionable efficacy. Many Japanese doctors seem to select anti-cancer drugs for their lack of side effects rather than efficacy because such drugs can be prescribed in large quantities for large profit, according to Dr Jonathan P. Bolton, an analyst at Schroder Investment Management (Japan) Ltd.

David Swinbanks



Nature 329, 575; 1987). The LSA was due to have opened last year, but construction delays pushed that date back to January.

According to Gunther Stent, who will chair the new department of molecular and cellular biology, no scientists were allowed inside the building until an opening cocktail party in January. Many of those who then toured the building were appalled by what they found.

Noise was the most noticeable problem, occasioned by the volume of air pumped through the ventilation system to yield 12 air exchanges an hour. The problem is evidently exacerbated by turbulence; air streams make right-angled bends as they pass through ventilation ducts.

Other problems have also come to light. Some shelving was mounted upside-down, air was drawn into fume hoods at such a velocity as to make them virtually un-

Canada's science funding

SIR—Your recent articles on “Science in Canada” (*Nature* 333, 717; 1988) clearly identified our biggest problem as the very low funding of research, and particularly industrial research and development, where we suffer from the branch plant nature of much of our industry. Why should a company carry out research and development at a subsidiary in Canada if it can do it more cheaply or conveniently at the parent plant in Chicago or Los Angeles? This problem is likely to become even worse if the so-called free trade agreement between Canada and the United States becomes law.

For many years, federal governments have recognized that Canadian science is seriously underfunded, and have promised remedial measures to bring the spending up to some figure, usually around 2.5 per cent of gross national product, within a few years. Results have, however, been negligible. The present government is no better than its predecessors; in fact, it may be worse, if only because it promises more, but with no more results. For example, the Prime Minister announced last winter that the government would spend an additional \$1,300 million on science and technology in the next five years. In fact, as pointed out by Bill Rompkey, MP (*Canadian Research/Biotechnology Canada*, Winter 1988), every dollar can be accounted for by cuts made elsewhere in science-related spending. The recent announcement that much of this money will be reserved for work related to the US space station may, in fact, mean less money available for other areas of science than now.

With respect to McGill University, I wish to make two points, one an interpretation of your figures and the other a correction. In your table on p. 723, you report federal research and development income for ten universities. To understand these figures, the sizes of the universities should have been included. For example, the University of Toronto received by far the largest amount (12.3 per cent), and is well recognized as running the largest research operation. McGill follows, with 8.6 per cent. However, when one takes into account that Toronto is twice the size of McGill, the figures take on a different significance. In fact, last year it was reported that, among Canadian universities, McGill was first in terms of research grants per staff, graduate students per staff and graduate degrees granted per staff. The second point, a correction, relates to the statements on p. 730. You refer to “... McGill University ... now one of Canada's best known and wealthiest universities”, and appear to draw a contrast by stating “The Université de Montréal has come through some difficult

times, hit by the economic depression of the early 1980s”. There was a time when McGill was relatively wealthy. However, after many years of chronic underfunding (according to its own formula, Quebec's Department of Education calculates that McGill is underfunded by about \$20 million per year), its free endowments have been exhausted to finance current operations. Reporting in April on improvements in Quebec university finances, Education Minister Ryan announced that with the exception of Concordia and McGill Universities and l'Ecole des Hautes Etudes Commerciales, which remain in a difficult financial situation, Quebec universities — the universities of Montreal, Sherbrooke, Laval and Quebec and Bishop's University — have attained financial equilibrium. Because in politics perception is more important than fact, your labelling of McGill University as “wealthy” helps to perpetuate a myth that reduces our chances of fair treatment even from the best-intentioned government.

E.R. BOOTHROYD

*Department of Biology,
McGill University,
Montreal, PQ, Canada H3A 1B1*

Graduate salaries

SIR—In the article “Degrees of salary and satisfaction” (*Nature* 334, 393; 1988), Jim Taylor draws attention to the comparatively low average salary for biochemists during their first six years in the job market compared to other science graduates, especially those in mathematics, computing and engineering.

Although later in the article the author states that “... the proportion of graduates obtaining further qualifications after their graduation is uncorrelated with their salary levels ...”, it is difficult to see how this can be so. From information in the same table, we see that 87 per cent of biochemists obtained postgraduate qualifications — a higher proportion than in any of the other sciences surveyed. Surely this means that for three to four years of their potential period of employment, most of these graduates will have been PhD students with a grant of £2,975 a year at current rates (£3,630 in London). This must have the effect of drastically depressing the average salaries obtained by these graduates in their first years of employment, placing them at an apparent disadvantage compared to their peers who went straight into employment after graduation.

H.M. KEIR

*The Biochemical Society,
7 Warwick Court, High Holborn,
London WC1R 5DP, UK*

UK science teaching

SIR—I am a practising science teacher and have some sympathy with the views of M.H. Dodson (*Nature* 333, 9; 1988). I was, however, surprised by the letter from Geoff Hayward and Martin Hollins (*Nature* 333, 698; 1988), which seemed to be attacking comments that had not been made. (Perhaps this is why Hayward and Hollins used the word ‘myths’ to describe these non-existent statements.)

Like good teachers, they organize their comments “First, he says ...” followed by a statement that Dodson did not make about education at school being “merely an apprenticeship for the real education that they will receive once they reach university”. Undoubtedly there are people who hold such views, but Dodson's letter does not suggest that he does. And in any case, is it not true that ‘A’ levels are “an apprenticeship” to other things, whether they are university studies, working in an estate agents, or being a policeman?

The second criticism of Dodson by Hayward and Hollins concerns standards. They refute his ‘suggestion’ that the standards of science education are high. “By any criterion this is simply not true”, they say. But I take the term ‘standards’ in the sense that Dodson uses it in the final paragraph of his letter to mean something about the level of academic attainment. Hayward and Hollins seem to be using it as a measure of how good the process of teaching is. The confusion over the terms used may mean that each is correct.

I agree with Hayward and Hollins that “University entrance requirements have distorted the educational system for too long”. But are they also saying that the standards of ‘science education’ are as they are (by implication, not very good) because of distortion resulting from the university entrance requirements?

If we pose the question “What is formal education for?”, there will be a number of answers. Some will apply to all pupils, while others will apply to relatively few pupils. Dodson's fears about ‘integrated science’ are concerned with one of the acknowledged aims of formal education. Arthur J. Kahn's letter (*Nature* 334, 466; 1988) makes it very clear what many science teachers fear about ‘integrated science’. Hayward and Hollins seem to be concerned with a different aim. The sooner we all realize that formal education attempts to fulfil several roles the better.

Finally, their letter seems to illustrate what is wrong with so much public debate in the United Kingdom. The debate seems to be absent; what remains is statement of views with scant regard to what the ‘opposition’ has actually said.

ROLAND DIXON

*20 Church Drive,
West Buckland, Wellington,
Somerset TA21 9LZ, UK*

Who knows how the brain works?

Three hundred scientists gathered in Cambridge, Massachusetts to listen, reflect and argue over how science is proceeding in answering questions about mind and brain. Alun Anderson and Joseph Palca report on Nature's 11th international conference.

Boston

"TEN years ago, to have had a meeting called 'How the Brain Works' would have been slightly disreputable. That it is taken seriously by psychologists, neuroscientists and computational scientists, signals in part a change in the times."

Those comments, made by philosopher Patricia Churchland, capture the intellectual flavour of last week's conference sponsored by *Nature* with the aforementioned ambitious title. The two-day meeting emphasized how approaches from the molecular level of the individual synapse to the global level of memory and perception show signs of moving together.

Cautious optimism for bridging the chasm between psychology and neuroscience rests on two foundations, according to Churchland. First, there are incredible new techniques for visualizing the molecular basis of behaviour, techniques that "would have made Sherrington green with envy".

But there have also been tremendous advances in computers and computing skills that have made it possible to construct functional models of parts of the nervous system. Neural models have shown that distributed control of the nervous system is computationally plausible, opening the door to a view of the brain containing multiple, independent, parallel pathways. "Being able to simulate such a system has made it possible to think of these things in a more palpable way", says Churchland.

The confluence of neuroscience and psychology has permitted some of the fundamental questions about the nature of mind to be asked in an experimentally testable way. Consciousness, the organization of perception, and the nature of meaning have lost some of their abstract qualities. Churchland points to intriguing experiments by Antonio Damasio in

patients with prosopagnosia — the inability to recognize familiar faces.

Although these subjects cannot identify pictures of famous people when asked who they are, they show autonomic changes — such as altered skin conductance — when shown familiar faces, suggesting that on some level the subjects do recognize the picture. Consciousness then seems to fall somewhere between visual recognition and recall.

Parallel pathways

Speaking at the conference, Damasio presented convincing evidence that it is possible to study these parallel pathways by performing cognitive studies on patients with specific brain lesions. New neuro-imaging techniques such as molecular resonance imaging and computer-assisted tomography now make it possible to identify accurately the parts of the brain damaged by disease.

Patients with lesions in the association areas for vision, for example, will retain the ability to perceive objects, but may show some peculiar deficit such as inability to perceive colour or to recognize faces.

More striking are data presented by Damasio on patient Boswell, a male subject who 14 years ago suffered extensive bilateral lesions to the higher association areas of the cortex. Were the brain organized from back to front in a unidirectional cascade of information processing, this patient should have totally disrupted perceptual skills. In fact, Boswell retains intact categorical knowledge about objects such as chairs, tables and clocks, learned before his brain injury, and can place them in an appropriate context. But he cannot learn new categorical objects, nor can he store any new declarative knowledge for longer than 40 seconds. Damasio argues from this that perceptual features

are extracted in early stages of visual processing, and the rest of the brain is involved in registering combinatorial codes through feedback.

Marcus Raichle presented another way to study the parallel pathways used in processing information in humans. Raichle uses positron emission tomography and short-lived radioisotopes to measure cerebral blood flow while performing processing tasks. By summing across trials and subtracting experimental from control signals, resolution can be improved to as little as 14 mm.

Flashing a word on a screen not surprisingly causes increased blood flow to the visual cortex. But for words presented aurally, not only are auditory areas stimulated but so too are certain other association areas, suggesting additional parallel processing of auditory information. More complex lexical tasks also make it clear that a sequential model from sensory input to lexical coding cannot be possible, and that some parallel activity must be occurring.

Parallel sensory pathways

Lower down in the visual system, psychophysical, physiological and anatomical evidence of multiple parallel pathways has been put together by David Hubel, working with Margaret Livingstone, among others.

Hubel described how the visual system splits into three pathways by the time it reaches the cortex, each with differing sensitivity to depth, colour, movement and orientation. The division originates in the retina and is detectable in the lateral geniculate bodies, the first brain nucleus to receive visual signals. Cells in the geniculate's parvocellular division are more sensitive to colour and have higher acuity, whereas those in the magnocellular division pick up contrast better and respond faster.

Underscoring the possibility of merging classical neuroscience with actual human behaviour, Hubel presented numerous striking visual illusions that confirm the division of labour (although many details remain controversial). The illusions are artificial situations where the information provided by one sub-system is missing, or conflicts with the information provided by another.

Pictures can be created that lose depth (signalled by the colour-insensitive,



Pat Churchland: incredible techniques. Antonio Damasio: convincing evidence. David Hubel: merging neuroscience and behaviour.



Crick: blasting algorithms. Mead: standard text. contrast-sensitive magnocellular system) when the colours in them are adjusted to the same intensity. Similar tricks can be played with movement. Hubel's relatively recent interest in human experimentation prompted Horace Barlow to quip "Welcome to the world of psychophysics".

But why does the visual system use multiple parallel mechanisms? An answer stressed by Vilayanur Ramachandran is that perception is "utilitarian". There is evolutionary pressure for parallel shortcuts that get the job done quickly. When a leopard springs, you will track where the leopard is, but ignore details of what its spots are doing. Ramachandran illustrated the point with visual illusions of "motion capture" in which dots providing the fine detail of a larger object are seen to move in the same direction as the object containing them, even if they do not. In other words, rules about the whole are blindly applied to visual details.

But if sensory pathways operate in parallel, how do they ever get back together to form the integrated image of the visual world we see with the mind's eye? Closing his talk, Hubel acknowledged that "we don't have the slightest idea. In fact we sometimes wonder if it is necessary to suppose that they get together at all. Could they not just be parallel and independent and not have any final common pathway?"

While conventional philosophers may be horrified to see the issue of central representation dispatched so mercilessly, Hubel's wry remark finds an echo in research from a quite different discipline.

Rodney Brooks designs mobile robots of all descriptions. One, the forerunner of a robot intended to travel over the rough terrain of Mars, is a six-legged insect-like creature that can crawl over a few piled-up telephone books. In designing his robots, Brooks has turned his back upon the traditional artificial intelligence approach that decomposes a robot control system into functional modules of perception, representation of the external world, task execution and motor control. His robots have no central representation or central system. Rather, they are a collection of competing behaviours, each in a separate activity producing layer, that connects perception to action directly. Building robots this way allows Brooks to add extra layers of control at will, while allowing

lower layers to continue functioning. Seeing a videotape of this robot under distributed control wandering around the laboratory in an apparently purposeful way, it is hard not to impute a central control system to it. That complex behaviour may have simple roots is a thought ethologists will be familiar with.

Models of the brain

Neural-network models must take much of the credit for putting the grand view back into neurobiology. But do these models really tell the biologist anything useful?

A feature of neural-network models that disturbs many biologists is the way the network is 'taught': actual output from a model system must be compared to desired output before the connections in the system can be adjusted slightly to reduce the error.

So far, modellers have preferred the 'back-propagation' method of error reduction. A three-layered network consisting of input, output and hidden units is capable of impressive results. Such networks have been built that can recognize words and extract shape from shading.

But modellers such as Geoffrey Hinton are aware that back-propagation is literally implausible in a biological system. It would be nice to have evidence that the process of gradual adjustment of connections in a network is going on, independent of a particular learning method. Such

evidence is at best suggestive: Hinton pointed out that 'gradient descent' systems share with biological systems the property that, after damage or forgetting, they will relearn the solution to a problem much more quickly than when they learn it from scratch.

Back-propagation models are also slow and do not scale up well to large nets. They will always suffer from the problem that changes in a synaptic weight in one part of the system are coupled to changes everywhere else through the effect on the error signal. One solution is that there should be many modular structures rather than one big network.

To impress, neural networks must do more than solve problems, they must also generalize well. Hinton showed that if there are too many units in the network it may simply turn into a 'look-up' table containing the correct answer to every possible question. A system is clever and generalizes when it has fewer units and is forced to discover the underlying regularities in problems.

How many units are ideal for a particular problem? David Rumelhart tackled this problem by designing a network that minimizes both the error in output and the system's overall simplicity (essentially the number of units and connections). Experiments with such networks show that they generalize well. But the combined error-and-simplicity minimization system will not find direct analogy in the brain. Rather, by showing how systems that do a particular computation can be well designed, it will throw light on what biological systems are doing (as well as aiding engineers in designing efficient networks).

Models maligned

Although model building has become an integral part of the computational approach to understanding neural functioning, there exists a deep-seated suspicion among some that model building is not so much science as engineering. A fractious Francis Crick took elaborate pains to point out that a scientifically informative model must be based on structures that actually exist in the nervous system.

Although neural modellers would agree that the precise mapping of model constructs onto neural architecture will require better understanding of just how that architecture works, they nonetheless argue that models themselves are useful objects for study. David Tank spoke out strongly in favour of distinguishing between an algorithm, in the sense that David Marr used the term, and the implementation of that algorithm in particular hardware. Both he and Terrence Sejnowski pointed out that the 'Hebb synapse' can be implemented in many ways — in a single synapse or in a circuit using heterosynaptic facilitation — indeed, the NMDA recep-

Nature's 11th international conference "How the brain works" was held on 28-29 September 1988 in Cambridge, Massachusetts. Sponsorship for the session on perception was provided by the Fidia Research Foundation of Washington, DC.

Chairmen: Emilio Bizzi (MIT), Max Cowan (Howard Hughes Research Institute), Francis Crick (Salk Institute) and Tomaso Poggio (MIT).

Speakers: Rodney Brooks (MIT), Antonio Damasio (University of Iowa), David Hubel (Harvard University), Eric Kandel (Columbia University), Geoffrey Hinton (University of Toronto), Stephen Lisberger (University of California at San Francisco), Carver Mead (Caltech), Richard Morris (Edinburgh University), Mark Raichle (Washington University), Vilayanur Ramachandran (University of California at San Diego), David Rumelhart (Stanford University), Terrence Sejnowski (Johns Hopkins University), David Tank (AT&T Bell Laboratories) and Shimon Ullman (Weizmann Institute).

Rapporteurs: Horace Barlow (Cambridge University), Patricia Churchland (University of California at San Diego), Charles Stevens (Yale University) and Terrence Sejnowski (Johns Hopkins University). □

tor might even be called a Hebb molecule.

Tank argued that regardless of precisely how a Hebb synapse is implemented in the nervous system's wetware, much can be learned by studying models of the computation algorithms using such a synapse. Crick took great exception to this approach, blasting algorithms as "just a branch of mathematics" and declaring analyses that do not capture biological details are not models but "caricatures".

Tomaso Poggio took a gentler attitude in accepting that there should be both a top-down approach "trying to characterize what the problems are, trying to see how they can be solved in principle, without worrying at first whether the machinery is biological or artificial . . . and a bottom-up approach, starting from the properties of neurons, and trying to build models of neural networks and see what they do". He cautioned, though, that "present models of neural networks may not take sufficient account of the biophysical properties of real neurons".

Top-down

Attempts by artificial intelligence researchers to programme computers to match the capabilities of the human visual system quickly reveal the enormous difficulty of the problems. Shimon Ullman has been trying to build machines that can recognize objects: the problem is essentially one of the correspondence of perception to memory. What makes it so difficult is that the same object has to be recognized when 'transformed': seen

describes mathematical techniques that make the correct transformation relatively easy. But such a scheme still suffers from the need for memory to store enormous numbers of representations and raises the question of how easily the system can be scaled up to deal with the real world.

Bottom-up

A unique approach that starts at the bottom and works up comes from Carver Mead, a charismatic figure who ten years ago helped revolutionize the way VLSI systems are designed. His book *Introduction to VLSI Systems* is the standard text on the subject. Soon to be published (and already circulating in draft version) is his next book *Analog VLSI and Neural Systems*. Mead ascribes part of the failure of digital computers to inform us about how the nervous system works to the brain's analogue method of computation. He aims to create analogue networks on silicon chips that mimic parts of the nervous system with the goal of providing a means for testing hypotheses about how bits of the brain work.

The idea of creating nervous systems in silicon is new; in part because only recently have engineering techniques emerged that are capable of building systems of the requisite complexity, and in part because not enough has been known of the basic organizing principles of the nervous system. Mead has begun his analogue work with "his feet firmly on the ground" by building the earliest percep-

Sejnowski predicted that learning in at least one vertebrate system will be understood at a similar level — if not in the same molecular detail — within the next five years. The vestibulo-ocular reflex (VOR), being studied in monkeys by Stephen Lisberger, ensures that the eyes remain stably pointing in one direction when the head turns, so that the image of the surrounding does not smear across the retina. The basic circuitry of the system and the locations of the nuclei containing the neural hardware, are known. That makes feasible the possibility of directly studying the synapses responsible for learnt changes in a defined behaviour in a vertebrate.

Study of the VOR will also open a window on the still-enigmatic cerebellum. As Lisberger showed at the conference, the cerebellum provides both on-line corrections to errors in VOR and more permanent long-term corrections.

Explosive progress is also being made on the hebbian synapse and what may be its molecular embodiment in the NMDA receptor. Charles Stevens predicted that the molecular basis of long-term potentiation (LTP) in the hippocampus (the key preparation in studying modifiability of mammalian central nervous synapses) will be understood within "a year or two".

But there is always something new. Sejnowski — who appears in alternate guises as theoretician and experimenter — surprised with results from a standard hippocampal preparation showing that long-term depression (LTD) of synaptic activity can occur as well as LTP. While LTP requires convergence of paired stimuli, LTD requires out of phase (anti-paired) stimulus. The molecular basis of the new effect (if that is what it turns out to be) is unclear; the chemical AP5 which blocks the NMDA receptor does not affect LTD, suggesting it has a separate mechanism.

Even if the molecular basis of LTP can be understood, it gives no guarantee that the function of the hippocampus will become clearer. Richard Morris has been building models of hippocampal function. He shows that AP5 interferes with the rat's ability to learn the position of an object but not its ability to learn to perform a straightforward visual orientation task. Morris sees a parallel between the spatial task and 'declarative' learning. In other words, the rat's ability to indicate where something is in space is analogous to the ability to name an object. Morris also sees parallels between the visual orientation task and procedural learning.

The analogy may be a little far fetched, given that the distinction between the two forms of memory was originally made to help classify amnesia in humans. But the spirit of Morris's enterprise, linking changes at the synaptic level to changes at the level of the learning of complex tasks, was what the conference was intended to celebrate. □



Sejnowski: many routes to Hebb synapse. Stevens: long-term potentiation near to solution.

from different positions, at different distances, under different lighting conditions and when partially occluded by other objects.

Researchers have attacked the problem by seeking for invariant properties of particular objects so that they can always be recognized, and by seeking to construct structural descriptions of objects in terms of their parts and the relationship between them. Neither approach has worked well outside 'toy' laboratory worlds.

Ullman has followed a separate approach in which objects in image and memory are matched by aligning them exactly. Before this is possible, compensation must be made for differences in transformation — an analogy is the way we have to rotate objects mentally to 'see' if they are exactly the same. Although the correct compensatory transformation might sound very hard to find, Ullman

tual processing systems. He has already built a silicon retina, that produces output similar to that of a vertebrate retina when light is shone on it. His latest work incorporates a silicon cochlea and builds analogue time-delay circuits that perform the same computation of intra-aural time delay used by the owl to localize the horizontal position of sounds.

Hot topics

With the power and value of brain models disputed, safer bets for the research classics of the next five years lie at the molecular end of neurobiology where the power of new techniques makes rapid progress certain. Already in a class of its own is the series of studies by Eric Kandel and his associates on the neuronal and molecular basis of learning (both associative and non-associative) and memory (both long and short term) in *Aplysia*.

Reproduction

New horizons in contraception

R.J. Aitken and M. Paterson

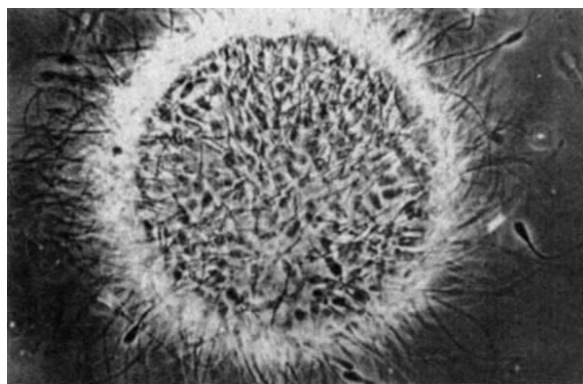
DURING the time it takes to read this article, about 1,000 new individuals will have been added to the world's population, 80 per cent of them in developing countries. In view of the poverty, disease and pollution which follow in the wake of this trend, the development of safe, effective contraceptives should surely be given the highest priority. Of all the methods currently being researched, the engineering of contraceptive vaccines must be the most exciting and appropriate approach to the problem. It is therefore of considerable interest to read on page 543 of this issue¹ the success of Paul Primakoff and colleagues in inducing infertility in guinea pigs by active immunization against the sperm surface antigen, PH-20.

There are two main targets for a contraceptive vaccine: fertilization of the ovum and implantation of the embryo. Although clinical trials have already begun² using a vaccine directed against a well-characterized product of the human conceptus, human chorionic gonadotrophin, the abortifacient mechanism of action implicit in this approach makes it unacceptable to many. A vaccine causing the disruption of sperm-egg recognition at fertilization would not suffer from this disadvantage as it would prevent the process of conception itself. Furthermore, there is considerable clinical evidence indicating that the development of immunity against gamete-specific antigens, without any detectable side-effects, is a feasible objective.

These clinical data derive from studies indicating that approximately 5 per cent of men and women attending infertility clinics show signs of immunity against sperm-specific antigens without any associated morbidity other than long-term infertility. The presence of antisperm antibodies in the serum or genital-tract secretions of either the male or female partner is associated with a significant reduction in cumulative pregnancy rates³⁻⁵, whereas reduction of antibody titres by immunosuppressive therapy is associated with a significant increase in fertility rates⁶. Further evidence of a causative relationship between the presence of antisperm antibodies and infertility has come from studies on the reversal of vasectomy. In approximately 70 per cent of men sterilized by this procedure antisperm antibodies can be detected⁷, the presence of which significantly diminishes the

chances of a successful reversal ever being performed⁸.

The mechanisms by which such antibodies depress fertility include complement-mediated immobilization of the spermatozoa⁹; immunoglobulin A-mediated disruption of cervical mucus penetration¹⁰; and inhibition of the recognition and membrane fusion events that comprise the fertilization process^{11,12}. Attempts to use antisera from patients



Sperm-zona interaction. The binding of spermatozoa to receptor sites on the *zona pellucida*, a translucent shell which surrounds the mammalian oocyte, constitutes the key gamete-recognition event in fertilization. Antibodies against specific components of the sperm surface can disrupt this stage of fertilization and thereby inhibit fertility. This property of anti-sperm antibodies is being used as the basis for engineering a contraceptive vaccine.

exhibiting idiopathic autoimmunity to sperm antigens to identify the sperm surface antigens responsible for the induction of infertility have been unsuccessful, mainly because of the complexity of the immune response¹³. Animal models, in which biochemical characterization of the sperm plasma membrane and the bioassay of sperm function can be readily achieved, have therefore been used.

Since the pioneering work of Landsteiner and Metchnikoff in 1899, the guinea pig has figured prominently in studies of the relationship between antisperm antibodies and fertility. Using polyclonal antibodies from autoimmunized male guinea pigs, Yanagimachi *et al.*¹⁴ demonstrated that the key event in *in vitro* fertilization disrupted by such treatment is the binding of spermatozoa to a translucent shell surrounding the oocyte, the *zona pellucida* (see figure). Sperm-zona interaction represents a major, probably the main, cell-recognition event in fertilization and is thought to involve the interaction of receptor sites (possibly glycosyl transferases) on the sperm plasma membrane with carbohydrate residues on the outermost surface of the

zona pellucida. Subsequent characterization of the sperm surface antigen involved in mediating *zona* recognition in the guinea pig was achieved by Primakoff¹⁵ using a monoclonal antibody directed against a guinea-pig sperm antigen, PH-20, associated with three proteins, a major component of relative molecular mass 64,000 (64K), a component of 56K and an endoproteolytically cleaved form comprising two subunits of 41-48K and 27K, respectively.

The location of this molecule and its precise role in mediating sperm-*zona* recognition are complex. In the intact sperm cell, the antigen is located in the posterior portion of the sperm head and in this position is unlikely to mediate the initial adhesion between the sperm plasma membrane and the *zona pellucida*¹⁶. In keeping with this conclusion, antibodies against PH-20 do not inhibit the initial adhesion between intact spermatozoa and the *zona* surface. Once bound to the *zona pellucida*, however, the sperm is stimulated to undergo a secretory event, the acrosome reaction, during which the PH-20 antigen suddenly becomes relocated to an anterior position on the sperm head (the inner acrosomal membrane). In this location, PH-20 is in an appropriate position to mediate sperm-*zona* binding and the disruptive effects of anti-PH-20 antibodies become readily apparent *in vitro*. The data reported by Primakoff *et al.* in this issue¹ indicate that the induction of active immunity against this antigen is also effective in disrupting fertilization *in vivo* and that active immunization is effective in both male and female guinea pigs.

These data provide convincing evidence for the feasibility of developing an effective contraceptive vaccine based on sperm-specific antigens. Moreover, they

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indicate that such a vaccine could control both male and female fertility and that the effects should be fully reversible. These data also emphasize the significance of sperm-zona interaction as the stage of fertilization that should be the focus for the development of a contraceptive.

A key issue raised by the paper in this issue¹ is whether antigens similar to PH-20 might be involved in mediating sperm-egg recognition in the human. The human spermatozoon also possesses surface antigens (HS 1A.1) which change in location during the acrosome reaction¹⁷, although there seems to be little relationship between this peptide and PH-20. The existing evidence suggests that the zona receptors on human spermatozoa com-

prise a group of small peptides with molecular masses of 15–18K¹⁸. Hence, although cross reactivity between human and guinea-pig sperm antigens undoubtedly exists¹⁹, there is, as yet, no clear evidence that PH-20 will exhibit contraceptive potential in the human. Nevertheless, the major impact of the paper of Primakoff *et al.* is unequivocally to demonstrate that the principle of an anti-sperm contraceptive vaccine is well founded. Characterization of the antigens on the surface of the human spermatozoon involved in sperm-zona recognition is now of immense importance. □

R. J. Aitken and M. Paterson are at the MRC Reproductive Biology Unit, 37 Chalmers Street, Edinburgh EH3 9EW, UK.

Materials science

Order in disorder

Robert W. Cahn

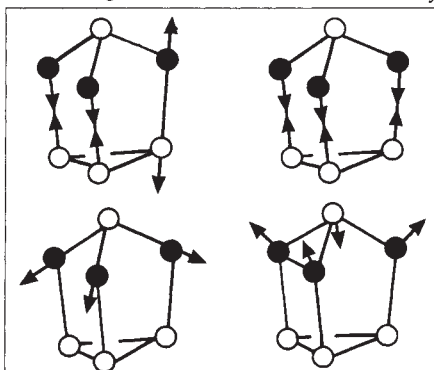
SINCE Zachariasen's seminal work¹ on the structure of oxide glasses it has been commonly accepted that such glasses are made up of a partially continuous network of SiO₄ tetrahedra, interspersed irregularly with alkali and alkali-earth metal ions. The experimental evidence for this proposition, mostly from X-ray scattering, has built up steadily over the years — for example, from the famous study of vitreous silica by Mozzi and Warren². Nevertheless, the second half of the proposition, what might be called the currant-bun model of the distribution of the metal ions, has never been established as firmly as the idea of the SiO₄ network. On page 525 of this issue³, Eckersley *et al.* marshal evidence indicating that the calcium ions in a calcium silicate glass have a first-neighbour shell ordered nearly as well as the oxygens around silicon atoms.

A common experimental trick for focusing structural information on a particular species in a glass is to determine scattering profiles for X-rays of two different wavelengths, one near an absorption edge for the element in question, so that scattering amplitudes are different; a differential Fourier transformation then gives specific information about the environment of the targeted atomic species. An even better way of achieving this end is to scatter neutrons, using two successive samples containing widely different isotopes of the target atom, with different scattering cross-sections.

Eckersley *et al.* used the latter approach with the calcium in their glass, and establish that, out to a distance of about 0.5 nm (2 atomic diameters), the environment surrounding calcium ions is closely similar to that in crystalline CaSiO₃ (which has a composition close to that of the glass examined). Specifically, about 85 per cent

of the oxygen ions nearest to a calcium atom in the glass must be disposed similarly to the corresponding ions in the crystal. A further implication of this finding, as the authors prove by careful calculations, is that calcium ions are not randomly distributed but have rather well defined, preferred separations. The venerable network model must thus be modified in the direction of enhanced order.

Although the network model is firmly



Four of the ten possible vibrational modes of the molecule P₄Se₃. The observation of these and other modes in the vibrational spectrum of P₄Se₃ glass permits Verrall and Elliott⁵ to infer the presence of molecular units in this system. (○, Phosphorous; ●, selenium.)

entrenched for oxide glasses based on silica, it is less so for metallic glasses (for example, Ti-Cu), over which a battle — by no means entirely semantic — has raged between the adherents of the dense-random-packing model introduced by Bernal and the microcrystallite model favoured by Gaskell⁴. For the latter, small groups of metallic atoms form virtually crystalline unit cells which are, of course, not disposed in parallel orientation to each other. Eckersley, Gaskell *et al.* make it specifically plain that their new findings

reported in this issue concerning the calcium silicate glass do not support the microcrystallite model for that glass.

A third category of glass is the molecular variety, where complete, undistorted individual molecules (monomers or polymers) are disposed in a non-crystalline array. These have hitherto all been organic materials, which are difficult to study with precision by scattering methods because the constituent atoms scatter X-rays and photons only weakly. Verrall and Elliott in a new study⁵ of a glass of composition close to P₄Se₃ establish that at least this inorganic glass is essentially molecular in its structure. Curiously, although the method they use again involves neutrons, in this case they were used to determine the vibrational density of states, not coordination shells of the material.

The use of spectroscopy, as distinct from scattering studies, to obtain information about glass structure is well established^{6,7}, especially for oxide and chalcogenide (sulphide, selenide and so on) glasses. What is new about Verrall and Elliott's study is the application of the method to a glass consisting, as it turns out, of well defined, distinct, inorganic molecules (see figure). The vibrational density of the states for the glass shows remarkably sharp peaks, normally characteristic of discrete molecules, and a comparison with Raman spectral peaks for crystalline P₄Se₃ shows an excellent correspondence. The observations⁵ even allow an estimate to be made of the nature of the intermolecular material when another P-Se glass, of divergent composition, is examined. Perhaps in due course a proper diffuse X-ray scattering study will be undertaken to establish just how, statistically, the molecules are disposed with respect to each other.

The conclusions of Verrall and Elliott are particularly interesting in view of current debates about structural issues in respect of chalcogenide glasses⁸. Thus, pressure-optical experiments on glassy GeS₂ established that this glass consists of an irregular array of two-dimensional layers rather than a three-dimensional polymeric array. The new work by Verrall and Elliott adds to the structural variety of germanium-based chalcogenide glasses. □

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Robert W. Cahn is in the Department of Materials Science and Engineering, University of Cambridge, Pembroke Street, Cambridge CB2 3QZ, UK.

Morphogenesis

Developmental decisions and pattern formation

Miranda Robertson

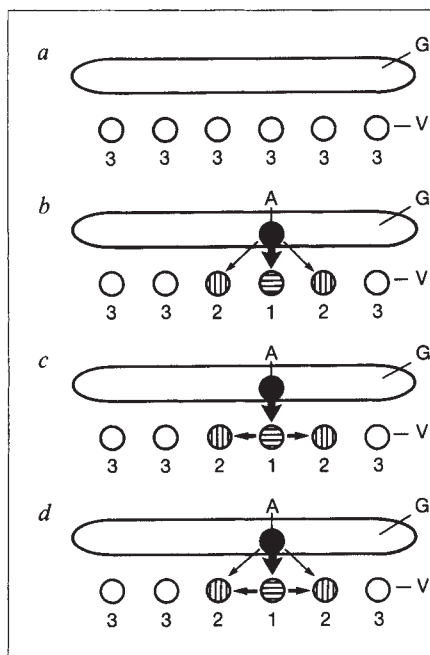
THE classical view of embryogenesis in the nematode worm *Caenorhabditis elegans* has been that developmental decisions are almost entirely dictated by lineage, with little if any contribution from the cellular interactions that are thought to be crucial to the formation of the body pattern in other organisms. (A distinction that Sydney Brenner used to characterize as European *versus* American — Europeans being defined by their ancestry and Americans by their neighbours.) The notion of a pre-programmed cell-autonomous progression from egg to adult would seem consistent with the remarkable invariance of the worm body plan; but recent research has increasingly eroded the peculiar position of worms in the annals of embryology; and two papers on pages 547 and 551 of this issue^{1,2} illustrate how invariance may be achieved in one critical feature of nematode morphology by a combination of two cellular interactions that together decide the fate of the vulval precursor cells.

One of the genes controlling this decision is already known to have striking similarities to a *Drosophila* gene with an analogous function; and given the phylogenetic distance between fly and worm, it may not be unreasonable to argue that developmental processes in nematodes are more likely to be a model for those of other organisms than a special eccentricity of the phylum.

The nematode vulva is generated from a group of six cells in the ventral epidermis (see figure). These differentiate in response to a signal from a single cell, the anchor cell, in the overlying gonad. The cell closest to the anchor cell is induced to become a primary cell, and the cells on either side of it are induced to become secondary cells. The remaining epidermal cells assume a tertiary fate by default: in the absence of the anchor cell, all six cells become tertiary. The three cell fates are distinguished by the pattern of division of the progeny cells which form the vulva. Mutations that perturb the determination of the six vulval precursor cells disrupt vulval morphology.

One target for such mutations is *lin-12*, a gene that is implicated in the differentiation of the secondary cells. This gene, whose complete sequence is reported¹ on pages 548–549 is believed from its primary structure to be a membrane-spanning protein whose putative extracellular domain comprises a repeated series of the motif characteristic of epidermal growth factor and of several cell-surface receptors,

while its putative cytoplasmic domain shares a repeated motif with the yeast cell cycle genes *cdc10* and *SW16*³. It shares this pattern of similarities, and other sequence similarities, with the *Drosophila* gene *Notch*⁴, which is implicated in the decision of embryonic ectodermal cells between a dermal and a neural fate: in extreme *Notch* mutants, all the ectodermal cells differentiate into neurons and the fly is overrun with neural tissue. In grass-



hoppers, in which an analogous developmental decision is made, it can be shown that the ablation of embryonic neural cells in a normal insect causes the ectoderm to follow the neural path⁵, from which it has been inferred that once a neural cell has differentiated it inhibits its neighbours from following the same fate. Thus *Notch* may be involved in the transmission of an inhibitory signal.

A similar interpretation of *lin-12* would be consistent with the mutant phenotypes but is less easily reconciled with the effects of manipulating the wild-type worm. Broadly speaking, loss-of-function mutants of *lin-12* result in the absence of secondary cells and an excess of primary cells, while in gain-of-function mutants primary cells are converted into secondary cells⁶. This is what might be expected if *lin-12* encoded a receptor for an inhibitory signal from the primary cell that prevented its neighbours from assuming a primary fate in response to the inducing signal from the anchor cell. The loss-of-

function phenotype would then be the consequence of loss of the receptor for the inhibitory signal, and the gain-of-function phenotype would be due to activation of the receptor in the absence of the inhibitory signal, preventing the expression of the primary fate in all cells.

Yet laser ablation experiments in which selected cells are deleted from the vulval precursor group suggest that the secondary cell phenotype is directly specified by the anchor cell. Thus any of the six cells of the group will become a primary cell if placed directly below the anchor cell; and a cell at a slight distance from the anchor cell will assume a secondary fate even in the absence of all the other cells in the group. This led Sternberg and Horvitz⁷ to conclude that both primary and secondary fates are probably induced by a graded

The six vulval precursor cells (V) with the overlying gonad (G) containing the anchor cell (A). *a*, If the anchor cell is eliminated by laser ablation, all six vulval precursor cells adopt a tertiary fate. *b*, *c*, Two possible mechanisms for generating the three precursor cell types in the vulval lineage. *b*, The anchor cell signal reaches the immediately underlying cell in high concentration, inducing a primary fate, and its two neighbours in lower concentration, inducing a secondary fate. The remaining cells in the group are out of reach of the signal and follow a tertiary fate by default. *c*, The anchor cell induces a primary fate in the immediately underlying cells, and the primary cell then induces a secondary fate in its two neighbours. *d*, A third possibility, consistent with all available evidence, is that the anchor cell induces both primary and secondary fates according to the strength of the signal, which depends upon the distance of the cell from its source; and primary differentiation of neighbouring cells is further prevented by a short-range inhibitory signal from the primary cell.

signal from the anchor cell, so that the closest cell, which gets the strongest signal, becomes a primary cell while the weaker signal to the neighbouring cells induces secondary differentiation.

With the report from Sternberg² in this issue, it becomes clear nonetheless that there is a lateral interaction between the primary and the secondary cells, and thus that the signalling system whereby these cell fates diverge is redundant.

This conclusion was reached on the basis of observations on a mutant, *lin-15*, in which the induction of the primary and secondary fates is partially independent of the anchor cell. In these mutants there are no tertiary cells, and all six cells are either primary or secondary. Sternberg's first observation was that two primary cells are almost never found next to one another in these mutants. But if he ablated all but one of the six precursor cells, as well as the anchor cell, the remaining vulval precursor cell invariably differentiated into a primary cell. Moreover by ablating some

of the cells in the group of six he could show that adjacent cells separated by a gap could both differentiate into primary cells.

Sternberg's observations may help to explain why, although the wild-type pattern of cells whose fate is controlled by *lin-12* is invariant, mutant phenotypes are more variable: it is not difficult to imagine that fluctuations in the strength of the anchor-cell signal could occasionally give rise to more than one primary cell in the absence of a mechanism for preventing it.

Moreover they seem to illustrate two of the mechanisms that might in general account for the generation of discontinuous patterns by a smoothly graded morphogenetic signal, such as is believed to determine the pattern of developing tissues in many systems. One possibility is a threshold response on the part of the cells to signal concentration; a second is lateral interactions between cells as they are sequentially induced. Both seem to

operate in the determination of secondary cell fate in the nematode vulval lineage.

It is however a source of some embarrassment that the two signals themselves so far remain an act of faith. If they exist, it should be possible to find mutants that lack them; or that suppress *lin-12* mutant phenotypes. It would be too bad if nematode morphogens proved as elusive as everyone else's.

For their help and suggestions on this short piece I am indebted to Jonathan Hodgkin and Robert Horvitz who, however, are not responsible for any errors or misstatements. □

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Miranda Robertson is Biology Editor of Nature.

Hydrothermal vents

Surprises of different kinds

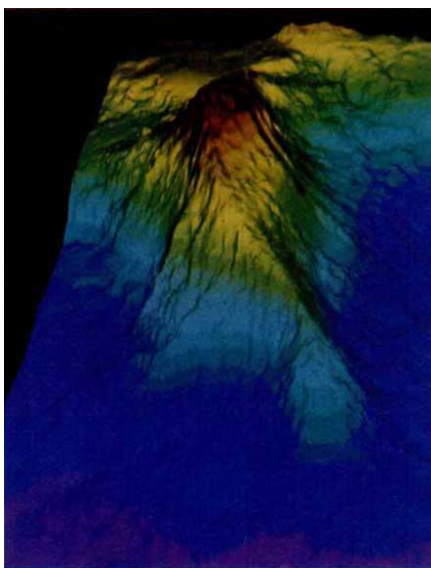
J.R. Cann and M.R. Strens

THE study of hydrothermal vents on the ocean floor has been pursued vigorously for only 10 years, so it is not surprising that new problems constantly arise. Some of our admittedly shaky ideas about hydrothermal venting have recently been challenged. Vents were not supposed to occur on slow-spreading mid-ocean ridges, but then they were discovered¹ in the mid-Atlantic where the spreading rate is only 1.5 centimetres per year. Vents appeared to flow at more or less constant rates, until the great outbursts called megaplumes were observed². Clearly, there are many more surprises yet to come, two of which are reported in the papers on pages 514 and 532 of this issue^{3,4}.

In the first of these papers³, Campbell *et al.* describe the chemistry of the waters of the Mid-Atlantic Ridge high-temperature vents discovered in 1985. These vents occur in much deeper water than the classic vents of the Pacific — 3,500 compared with 2,500 metres — on much slower-spreading mid-ocean ridges and in much more fractured terrain. The sulphide deposits around the Atlantic vents are apparently much larger than those around the Pacific vents⁵, suggesting a greater age, or at least that a much greater mass of hot water has passed through the Atlantic vents. All of these are good reasons in theory why the chemistry of the solutions should be very different.

The surprise reported by Campbell *et al.* is that the Atlantic solutions are very

similar to those of the Pacific. The elements that are stripped rapidly from rocks, such as lithium, occur at about the same concentration even though in more mature systems these elements would be expected to be depleted. Components predicted to be pressure sensitive, such as SiO₂ and the iron-sulphur ratio, are well within the range of values observed in Pacific vents. Even the temperatures of



Loihi Seamount, Hawaii, viewed from the south. Colour bands represent 100 metres. The image was produced by NECOR Sea Beam Development Center at the University of Rhode Island. (Courtesy of D. M. Karl.)

the venting waters are about the same in the Pacific and the Atlantic, reaching high values of about 350 °C, although the higher pressure would, under conventional theory, allow temperatures to rise to higher levels because of the effect of pressure on the thermodynamic properties of water. So clearly the theory (or theories) needs a careful examination. Campbell *et al.* make a start on the large amount of reconstruction that is necessary, but it seems likely that some profound new insight is required to explain their result.

In the other paper in this issue⁴, Karl *et al.* produce a different sort of surprise. Loihi Seamount (see figure) is the newest Hawaiian volcano, still 1,000 metres below sea level off the south-west coast of the island of Hawaii. It is very active volcanically, and has already provided a magmatic surprise in its eruption of a wide range of types of lava (traditional igneous theory would have predicted monotonous olivine tholeiites).

Observations of extinct sea-floor volcanoes near mid-ocean ridge axes had suggested that very active, high-temperature springs associated with large sulphide deposits might be characteristic of isolated, active volcanoes. But Karl *et al.* find that the hydrothermal activity on Loihi is very different. The springs are emitting water at about 30 °C, which is strongly enriched over sea water in barium, SiO₂ and particularly in iron, manganese, methane and dissolved CO₂ and bicarbonate. The solutions appear oxidizing, with abundant sulphate and no detectable sulphide. Associated with the vents are deposits of iron oxides and smectitic silicates reminiscent of the deposits that form on older crust near to the Galapagos spreading centre.

The source of the carbon is not at all clear. It is about 150 times greater in abundance than that in the ambient sea water, and it is difficult to see how such concentrations of CO₂ can be produced in the fluid phase alone. Perhaps it represents a contribution to the hydrothermal activity from volcanic gases, or perhaps the rocks with which the solution is reacting are abnormally carbon-rich. Such relatively acid, moderately oxidizing, CO₂-rich solutions have been suggested as a means of transporting gold⁶ and uranium to yield ancient ore deposits. Have the hydrothermal deposits from Loihi been analysed for these elements? □

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J.R. Cann and M.R. Strens are in the Department of Geology, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU, UK.

Solar System

A violent birth for Mercury

Glen R. Stewart

LESS than one-tenth as massive as either Venus or Earth, Mercury has an exceptionally high density, indicating that it has an iron-to-silicate mass ratio of about twice that of the other terrestrial planets. One way to produce such an iron-rich body would be first to form a body twice as massive as Mercury, melt its interior so that it forms an iron-rich core, then strip off the silicate mantle by a catastrophic collision with another slightly smaller planet. Implausible as such a sequence of events may seem, new numerical simulations of planetary-scale collisions performed by Benz, Slattery and Cameron¹ suggest such a procedure can yield a planet with the composition of Mercury. Furthermore, within the context of a currently popular model, simulations indicate that such catastrophic collisions may have been common during the final stages of planetary accumulation²⁻⁴.

Before the systematic reconnaissance of the planets by unmanned spacecraft, the popularity of any scheme for the origin of the Solar System was largely determined by its aesthetic appeal. By current standards, a credible scientific theory is required to predict observable consequences, so that one may discriminate between various hypotheses in an objective way. A sound theory of planetary formation should predict the observed masses of the planets, their bulk chemical compositions, the spacing between their orbits and perhaps the number of their moons. None of these planetary attributes can be explained with any certainty, but the important physical processes that probably led to their formation have begun to be identified.

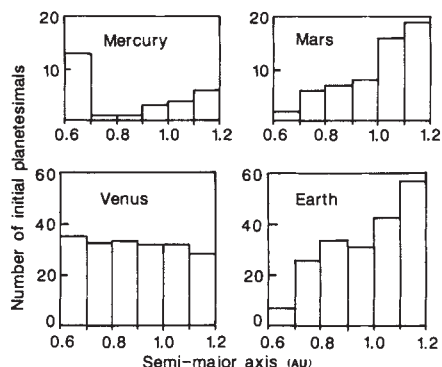


Fig. 1 Histogram of initial semi-major axes (in astronomical units) of the planetesimals that accreted to form the four inner planets (from ref. 4). Wetherill's computer simulations show that each planet contains material initially from a large range of orbits, destroying some (but not all) of the original radius-dependent fractionation effects in the condensing solar nebula.

Previous explanations for the unusual composition of Mercury have relied on the chemical fractionation of material that originally condensed out of the hot solar nebula, the disk of primordial gas that surrounded the young Sun, near Mercury's orbit. If the nebular temperature varied inversely with distance from the proto-Sun, then equilibrium condensation

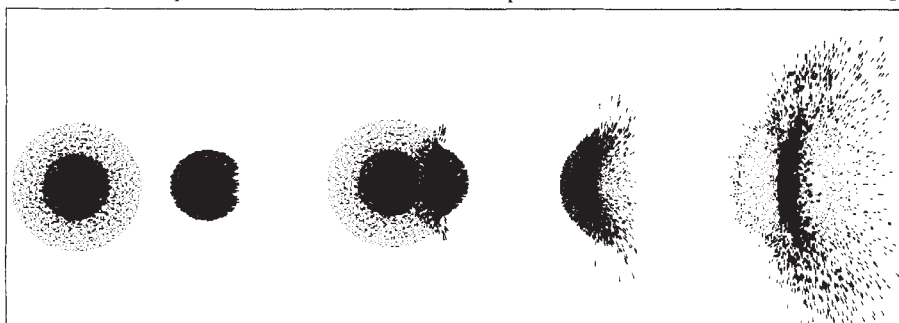


Fig. 2 Frames (at -1, +2.3, 7.7 and 41.7 minutes after impact) from the computer-generated film of Benz *et al.* showing a catastrophic collision of a proto-Mercury that results in a body similar to Mercury. (The last two frames have been scaled down by factors of 2 and 4, respectively.) Most of the silicates are ejected beyond the gravitational pull of the proto-planet, but the molten sheet of iron core eventually collapses to form the dense planet. (From ref. 1.)

favoured iron-rich condensates at Mercury's orbit, which is closest to the Sun⁵. Alternatively, if the comparatively small mass of Mercury is a consequence of mass loss by small particles spiralling into the Sun because of gas drag in the solar nebula, low-density silicate particles would be preferentially lost from Mercury's orbit before its accumulation⁶.

A stringent requirement for both of these models is that material at Mercury's distance from the Sun must remain isolated from the bulk of the material destined to be incorporated into terrestrial planets during the 10–100-million year timescale required for their accumulation from sub-planetary-size bodies. The idea that each planet grew from its own isolated feeding zone centred on its present distance from the Sun was popular until about 10 years ago. The suggestion that Mercury-size objects would be subjected to catastrophic collisions was considered highly improbable at that time.

Conventional wisdom began to shift away from the idea of isolated feeding zones once computers became available to simulate the final stages of terrestrial planet accumulation from a swarm of several hundred colliding, lunar-size planetesimals. Ground-breaking calculations, done primarily by Wetherill²⁻⁴, show that lunar-size planetesimals gravitationally perturb each other into crossing orbits. They thereby achieve relative velocities comparable with the escape

velocity required to break free of the gravitational pull at the surface of a typical planetesimal.

As planetesimals grow through collisions into planet-size objects, their mutual velocities keep pace with the escape velocity of the largest bodies in the swarm. At these velocities, collisions among the smaller bodies often lead to disruption rather than aggregation, whereas growth of the largest bodies is favoured by their larger cross-sections and their greater resistance to collisional disruption. Bodies near the size of Mercury are marginal cases, with collisions just about as likely to strip off material as to add to the final mass

of the planet. In these simulations, a typical body's orbit is likely to wander randomly over much of the zone now occupied by the terrestrial planets. As a result, the chemical fractionation associated with the heliocentric distance at which the planetesimals were originally formed is substantially reduced (Fig. 1).

Calculations of this sort lent respectability to the idea that the Earth's Moon was formed by an object perhaps as large as Mars colliding with the proto-Earth, lifting a fraction of its silicate mantle into Earth orbit⁷. Benz and his collaborators first applied their numerical simulations of planetary collisions to test^{8,9} this model of lunar origin and found that an iron depleted moon could result from such an event. In the case of a giant collision of a body with Mercury¹, the results are more catastrophic, with much of Mercury's mantle completely escaping from the planet's gravitational influence (Fig. 2). One obvious problem is that although Mercury initially becomes iron-rich, the disrupted silicate material is distributed in orbits similar to Mercury's and could eventually re-accrete onto Mercury to negate the effect of the collision.

Benz *et al.* argue that if most of the ejected material consists of sub-centimetre-size grains, then the orbits of these grains will evolve away under the influence of solar radiation pressure (the Poynting–Robertson effect) before Mercury can sweep them up. On the other

hand, if the proto-Mercury was in an orbit crossing the orbits of the much larger proto-Venus or proto-Earth at the time it experienced such a catastrophic collision, then most of the ejected material would be accreted by Venus and Earth. Wetherill's planetesimal simulations support the latter possibility.

Of course, none of these results provides conclusive evidence that the planets actually formed in this way. Numerical simulations of colliding planets must resort to drastic simplifications of an extraordinarily complex event, even with the power of a supercomputer. The relevance of the planetesimal simulations will remain in doubt until one can make a convincing case that there really was a starting swarm of lunar-size bodies. It is entirely possible that catastrophic collisions

between planetary-size bodies will lose favour once the earlier stages of planetary formation are better understood. For now, it will be interesting to explore further the consequences of what has come to be called the giant-impact hypothesis. □

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Glen R. Stewart is in the Laboratory of Atmospheric and Space Physics, University of Colorado, Boulder, Colorado 80309, USA.

Alzheimer's disease

Paired helical filaments and the cytoskeleton

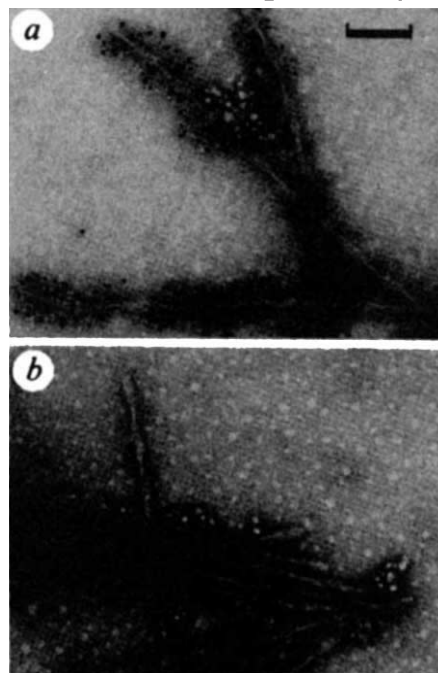
Brian H. Anderton

SENILE plaques and neurofibrillary tangles are the two principal abnormalities in the brains of people with Alzheimer's disease. The proteins in these abnormal structures, together with their normal counterparts, are gradually being elucidated. The amyloid protein comprising the senile plaques has been discussed recently in News and Views¹⁻³, and Claude Wischik and colleagues now report⁴⁻⁶ the isolation of a protein from paired helical filaments, the main components of neurofibrillary tangles. The protein turns out to be a fragment of the microtubule-associated protein called tau.

Massive accumulations of paired helical filaments (PHFs) occur in neurons of different areas of Alzheimer's brains, including those that are implicated in memory, such as the hippocampus. Formation of a neurofibrillary tangle impairs or destroys the functioning of the neuron. The new data of Wischik and collaborators provide firm evidence for the conclusion, already deduced from immunocytochemical studies, that the paired helical filaments are at least partially composed of cytoskeletal proteins (see ref. 7). Although Wischik and collaborators show that part of the cytoskeletal protein tau is tightly associated with the inner-core region of the PHFs, they estimate that up to 90 per cent of the mass of the filament core has yet to be identified⁸.

Wischik and collaborators had already shown⁸ that PHFs are built of a double-helical stack of transversely oriented subunits, each subunit having three globular domains. PHFs, or at least a fraction of

them, are remarkably insoluble, which has made their isolation as a homogeneous fraction virtually impossible. Antibodies against several cytoskeletal proteins, including tau as well as ubiquitin (a protein involved in the non-lysosomal degradation of abnormal intracellular proteins) 'decorate' insoluble PHFs *in vitro*, thus demonstrating that these pro-



Antibody labelling experiments on paired helical filaments with anti-tau antibodies. *a*, filaments that have not been treated with pronase show strong labelling; *b*, no labelling is seen in treated filaments. Scale bar, 100 nm. (From ref. 6.)

teins are constituents of PHFs. Wischik and colleagues have argued that tau and ubiquitin are located only in the periphery of PHFs, the region that appears fuzzy and amorphous in the electron microscope.

In their new work⁵, they therefore treated PHFs with the proteolytic enzyme pronase to remove the fuzzy coat. They then solubilized several polypeptides from the pronase-treated PHFs, which must be derived from a more central part of the filament which they call the core. Using a monoclonal antibody raised against the core, together with a chemical ligand, the details of which they do not provide, they show that two abundant peptides solubilized from the pronase-digested filaments, of relative molecular mass 9,500 (9.5K) and 12K, are labelled by the ligand and the antibody and hence must be derived from the core.

The amino-acid sequences of these peptides have some common regions. Wischik *et al.*⁴ synthesized mixed oligonucleotides and so isolated a complementary DNA corresponding to the parent molecule of the two PHF peptides. This precursor molecule is 352 amino acids long and is very similar to the recently published⁹ amino-acid sequence of tau purified from mouse microtubules.

Do PHFs contain only fragments of tau or does pronase treatment produce the 9.5K and 12K peptides from an intact or at least from a larger piece of the tau protein in the filament? The latter explanation seems the most likely as Wischik *et al.*⁶ find that two polyclonal antibodies against tau decorate untreated but not pronase-treated PHFs (see figure). Their monoclonal antibody against the core, on the other hand, preferentially labels pronase-treated PHFs. Because the 9.5K and 12K peptides overlap and come from the central region of the amino-acid sequence of tau, this region is presumably closest to the centre of the core whereas other parts of tau are more peripheral.

Ken Kosik and colleagues have now found that the bulk of the complete tau sequence is in PHFs¹⁰. They have mapped the approximate locations of the epitopes for five tau monoclonal antibodies that also decorate insoluble PHFs. The most amino-terminal epitope occurs between residues 61 and 90 of the amino-acid sequence, the most carboxy-terminal epitope is between residues 315 and 352, and the other monoclonal antibody epitopes are in between. Perhaps, therefore, both the amino- and carboxy termini of the tau polypeptide are further from the PHF core than the central region represented by the 9.5K and 12K fragments, the 9.5K peptide being identical to the tau sequence between residues 211 and 297.

Wischik *et al.*⁶ next asked whether the 9.5K fragment comprises the entire core. They measured in the scanning transmission electron microscope the mass per unit

size of tobacco mosaic virus. They conclude that, in the pronase-treated filaments, the three-domain subunit has a mass of about 100K and so, for a stoichiometry of one, the 9.5K tau fragment leaves 90 per cent of the subunit unaccounted for. This fraction is probably not composed of other tau molecules.

No doubt further analysis with the other components of PHFs, such as ubiquitin, will eventually resolve this discrepancy. It must not be forgotten that it is important to identify differences in the filament proteins in Alzheimer's brains compared with their normal location within the filaments. There are also other ultrastructural changes in PHF-bearing neurons that need to be investigated at the molecular level. It has been observed¹¹ that when PHFs are present, the normal cytoskeleton of microtubules and neurofilaments frequently disappears, and yet it is only tau and possibly also fragments of neurofilaments and microtubule-associated protein-2 that seem to end up in PHFs. What happens to the rest of the cytoskeleton?

A disorganized cytoskeleton appears to be even more widespread than the distribution of PHFs and as such may precede the appearance of PHFs in Alzheimer's disease. Microtubules are abnormally orientated, closer packed and fewer in number in a proportion of dendrites¹². The distribution of immunoreactivity to tau in Alzheimer's brains is also abnormal in regions other than PHFs. Tau antibodies usually stain only axons, but in Alzheimer's brains the cell body and dendrites of many neurons are also stained and the staining patterns show many dystrophic neurites in excess of what is believed to result from the presence of PHFs⁷. PHFs might therefore be the end stage of a disrupted neuronal cytoskeleton.

There is some evidence¹¹ of a close relationship between abnormal multilamellar membrane configurations and PHFs inside affected neurons. Gray and colleagues propose¹¹ that PHFs are produced by the aggregation of integral membrane protein(s) as the membranes disintegrate in damaged neurons. Thus, the missing 90 per cent of the core could be derived from a membrane protein. □

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Brian H. Anderton is in the Department of Immunology, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE, UK.

Archaeology

A trade not for gentlemen

Richard Hodges

"My dear chap, I know nothing whatsoever about Dark Age trade, or at any rate no more than befits a gentleman", says Gerald Middleton, professor emeritus of early mediaeval history in the satirical novel¹ *Anglo-Saxon Attitudes*. In the 1950s, historians studied constitutions and christianity, not economics. In the main this was because written sources failed to shed much light on trade, and concentrated instead on political and religious affairs. Archaeology offered the only promise of redressing this historical problem, as Dunning showed² when in 1956 he



Three of the many sceattas found in the Southampton area. These examples have a moustached face surrounded by eight or nine roundels on the obverse side (top), and on the reverse side (below) a bird walking right with raised wing and head lowered as if pecking. (From ref. 3, courtesy Southampton City Council.)

drew attention for the first time to the wealth of Dark Age material from the little-known 1946-47 rescue excavations in Hamwic, the Anglo-Saxon name for the town of Southampton on the south coast of England. Even so, few historians were convinced by the data. Thirty years later, with the publication³ of the coins and pottery found in Hamwic, many 'gentlemen' historians will now be compelled to take fresh account of this enigmatic aspect of the early Middle Ages.

The new monograph³ reveals the huge volume of archaeological data gathered at Southampton since 1946. Hamwic itself, it is often claimed, is the first English town. It was founded in the late seventh century in an age when market towns did not exist, and trade was regulated by the kings of the Anglo-Saxon tribes at their courts. Archaeologists at Southampton have shown⁴ that, on the basis of the 5 per cent excavated sample, the new planned town covered an area of about 45 hectares with a deep perimeter ditch. The excavations show that eighth-century Frankish traders assembled near the riverside, while the insulae within the gridded street network were occupied by craftsmen who made pottery, metalwork, bonework and a great range of commodities.

The very different studies reported in ref. 3 of the coins and pottery from the long-running excavations reveal the different spheres of Dark Age trade. The coins, for example, throw light on royal administration of the trade and indicate the main groups engaged in commerce with the West Saxons from what is now Germany. By contrast, the imported pottery provides some impression of the origins of the merchants who came to the port, while the distribution of crude, locally made pottery indicates the regional influence of Hamwic's lower-ranking craftsmen.

D.M. Metcalf's study (page 17 of ref. 3) of the coins found in Hamwic reveals that about half of the eighth-century coins (types BMC 39, 48 and 49) were probably minted in the port. A bewildering variety of dies were used to stamp these sceattas (small pennies made from pellets of silver; see figure). Metcalf estimates that 170-260 obverse dies and as many as 400 reverse dies were used to stamp the most prolific local type of sceatta, BMC type 49. As each die could be used 10,000 times, Metcalf claims that millions of eighth-century coins were in circulation in the town. Interestingly, almost none of these types has been found outside Hamwic. This suggests their use was restricted to the town, perhaps as a kind of taxable token or the equivalent of the coupons issued by West Saxon kings. Coins issued by other Anglo-Saxon and continental kingdoms at this time show that West Saxon monetary policy was unusual. About 16 per cent of the other coins were issued by Frisian mints, and may reflect the activities of the celebrated traders from this region. These traders are known to have been operating as far north as Jutland at this date and it is through them, Metcalf believes, that the small percentage (8 per cent) of Danish sceattas decorated with 'Wodan monsters' may have come. About 24 per cent of the coins were minted in other English kingdoms. Sceattas from Kent and southern Mercia make up the greater part of this group, although there is a solitary coin from Northumbria.

Coin finds are notoriously difficult to interpret. But in this case the 129 eighth-century sceattas cannot be ignored. In particular, the find suggests that the West Saxons had a highly regulated currency of their own, and that they were engaged in trade with the two neighbouring Anglo-Saxon kingdoms as well as with traders on the European continent. But the discovery of the high percentage of Frisian and Danish sceattas is intriguing in the

light of Jane Timby's analysis (page 73 of ref. 3) of the pottery found at Hamwic. About 18 per cent of the overall pottery assemblage, Timby shows, is imported. These wares, however, are mainly domestic pots that merchants brought with them to use while they traded in the town. Very few of the imported pots seem to have been traded in their own right. The percentage of imported pottery, in other words, is close to the percentage of imported coins.

However, the imported pottery in Hamwic came from northern France, the Paris basin, the Loire Valley and even as far away as Alsace. Less than 1 per cent of it comes from the north-west European Low Countries or the Rhenish areas in which the Frisians monopolized long-distance trading. The Frisians, it seems, tended to dominate a trading network covering the North Sea, stretching from East Anglia in England to Denmark, and left the English Channel to merchants from French ports like the recently discovered Quentovic in the Pas-de-Calais⁶⁻⁷. In these circumstances, Metcalf may be wrong to interpret the continental coins as evidence of Frisian merchants in Hamwic. Instead, it seems likely that traders from somewhere like Quentovic may have obtained these coins in dealings with the Frisians, and discovering them an unacceptable currency in France, found that they could be used in Hamwic.

The local pottery reflects the more primitive side of the Dark Ages. Unlike France and Germany, where wheel-thrown wares continued to be made using techniques that had existed since Roman times, the Anglo-Saxons manufactured only hand-made wares. At Hamwic the earliest wares occur in only a limited range of rudimentary cooking-pots, but a fine sandy clay was used, providing a burnished finish. A few of these pots were stamped using intricately ornamented bone stamps like those used by the potters who made the cremation urns of the earlier pagan Saxon period. But even this local tradition seems to have died out. Instead of attempting to emulate the wheel-thrown Frankish pots imported by the merchants into the town, Hamwic's potters turned out an increasing number of wares poorly tempered with local flint and calcite. It can only be concluded that the potters were forced to use poorer materials, including a lot of rough temper, to keep up with the increased demand for their wares. Paradoxically, like the scat-

tas, the fine sand-tempered wares have not been found outside Hamwic, whereas the poorer quality types occur in various excavated rural settlements around the Hampshire basin.

The new monograph³ shows that there is still much to be learnt about Dark Age trade. Evidently, as the English developed their first towns, they were laying the

foundations of the mediaeval economy through their trade connections. Studying the changing rhythms and patterns of this trade will be important if 'gentlemen' (and others) are to come to terms not only with the economics of this period but with the early constitution of the English nation. □

Richard Hodges is director of the British School at Rome, Via Gramsci 61, 00196 Rome, Italy.

Nuclear physics

Signs of variable nucleon size

A. W. Thomas and J. E. Thomas

IN the traditional understanding of nuclear structure, the nucleus consists of protons and neutrons (nucleons) interacting in pairs. The charge distribution of a nucleus is calculated by folding the spatial distribution of the protons together with the charge density inside the proton itself. Over the past few years this view has come under intense scrutiny. There is evidence from several experiments that suggests that the properties of free nucleons are altered inside a nucleus. Data from high-energy muon scattering on nuclear targets taken by the European Muon Collaboration¹ (EMC) at CERN suggest that the nucleon could swell by up to 15 per cent in iron². And Siegel *et al.* demonstrated³ that problems in interpreting data, taken at Brookhaven National Laboratory, on the elastic scattering of positive kaons (K^+ mesons) from carbon⁴ could be overcome if the nucleons were larger inside the nucleus than in free space. Now Brown, Siegel and collaborators⁵ propose an interesting and controversial mechanism for the size increase needed in the K^+ reaction.

Particle scattering, originated by Rutherford, is the usual technique for discovering the structure of nuclear particles. 'Deep inelastic' scattering of high-energy electrons revealed the quarks that comprise nucleons; and muons, heavy electrons, are the probe used by the EMC. The Brookhaven experiments of Marlow *et al.*⁴ use kaons which are themselves composite particles containing a quark and an antiquark. The details revealed depend in part on the interaction involved — electroweak or strong — which is typically envisaged as the exchange of a 'virtual' particle (Fig. 1).

In the treatment of Brown *et al.*⁵, the K^+ -nucleon interaction is carried by the exchange of virtual vector mesons, the rho (ρ) and omega (ω) (Fig. 1a). With a rest-mass energy $m_v c^2$ of 800 MeV (m_v is their mass, c the speed of light), almost equal to that of the proton, these cannot be created as real particles because of energy conservation. But because in quantum mechanics energy need not be conserved for times τ of the order of $\hbar/m_v c^2$ ($\hbar$ is Planck's

constant), these particles can exist as temporary, or 'virtual', entities.

The range of this force is limited to a distance of the order of $c\tau$ which is proportional to $1/m_v$ and is about 0.25 fm. (1 fm = 10^{-15} m and is a characteristic size in nuclear and particle physics: the oxygen nucleus has a radius of just over 2 fm and the lead nucleus, 6 fm.) To be more pre-

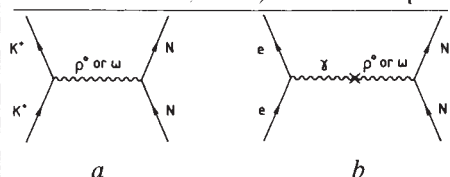


Fig. 1 a, The kaon-nucleon (K^+ -N) scattering mechanism studied by Brown *et al.*⁵. (Time increases up the page.) Quantum mechanics permits the exchange of a virtual vector meson (ρ or ω) over a distance inversely proportional to its mass. Energy and momentum are exchanged in this way. b, The corresponding mechanism for electron scattering, which measures the nucleon charge density. The electron (e) emits a virtual photon (γ) which has the same quantum numbers as a vector meson. According to vector-meson dominance, the photon interacts with the nucleon by converting into a ρ or ω before being absorbed.

cise, the root-mean-square radius of the K^+ -nucleon force, R_{K+N} , is given by $R_{K+N}^2 = 6/m_v^2 + \langle r_{core}^2 \rangle$, where $\langle r_{core}^2 \rangle$ is the size of the 'core' of the nucleon.

This gives the range of the K^+ -nucleon force in free space. Brown and co-workers then suggest that inside a nucleus, in the presence of other nucleons, the vector meson masses are lowered. The extent of this decrease is controlled by a parameter λ such that in the centre of a heavy nucleus $\lambda=0.2$ would mean a 10 per cent decrease, $\lambda=0.4$ would mean 20 per cent and so on. From the equation, if the core size of the nucleon is about equal to the vector meson contribution (and does not change), a 20 per cent decrease in m_v means a 10 per cent increase in the K^+ -nucleon range so that the apparent size of the nucleon increases by the same amount. This is exactly the sort of increase which Siegel *et al.*³ found empirically would enable them to fit the K^+ - ^{12}C scattering data of Marlow *et al.*⁴ (Fig. 2).

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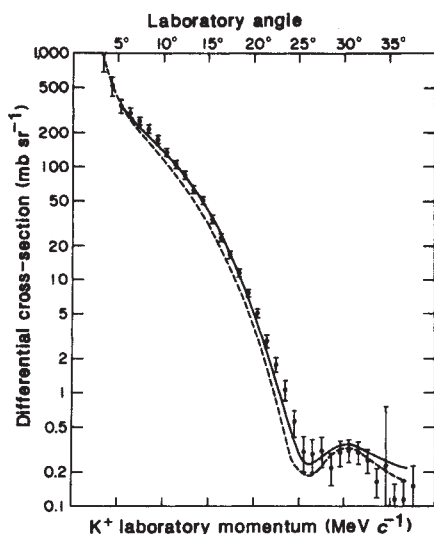


Fig. 2 Comparison with the data of Marlow *et al.*⁴ of theoretical calculations of K^+ elastic scattering from carbon before ($\lambda = 0$, broken line) and after ($\lambda = 0.2$, solid line) allowing for a possible change in the size of the nucleon.

The decrease in the vector meson mass is easily understood. In a simple quark model, the ρ and ω are made of a loosely bound quark-antiquark pair. Because they are weakly bound, m_v is approximately twice the mass of each quark ($2m_q$). At very high densities, say five to ten times the density of the most dense nucleus, quantum chromodynamics (the most widely accepted theory of the strong interaction) predicts a transition to a phase in which m_q is zero. In one widely used model⁶ the variation of m_q between the low- and high-density limits is approximately linear⁷, naturally giving a 10–20 per cent decrease in m_v at normal nuclear matter density.

Of course Fig. 2 shows calculations for K^+ scattering from a nucleus, not a nucleon. Can one be sure that there is no theoretical problem in computing the K^+ -nucleus interaction? Although Brown *et al.* note that there are some corrections still to be calculated, the answer is probably yes. For many years, nuclear physicists have known that the K^+ is an ideal probe of nuclear structure — a 'strongly interacting electron'. The reason is that the quark structure of the K^+ (it contains an antistrange quark) does not allow it to resonate with a nucleon in the way that (say) the related pion does. In consequence the K^+ -nucleon cross-section is small and varies slowly with energy. With a mean free path of typically 5 fm or greater, a K^+ will rarely suffer more than one collision with a nucleon in passing through a carbon nucleus. This limited number of collisions gives one great confidence in the theoretical calculations of the K^+ -nucleus interaction (the microscopic optical model).

The lack of K^+ experimental data is attributable not to any lack of enthusiasm but to the difficulties of making usable

beams. Like the pion, the K^+ only lives 10^{-8} seconds before decaying into pions which then contaminate the beam.

The experiment of Marlow *et al.*⁴ used the K^+ beam at the Brookhaven AGS (alternating gradient synchrotron) which is the best available. In addition the measurements were carefully performed. Nevertheless, there could be significant systematic errors — up to ± 17 per cent in the absolute normalization of the data⁴. Only new experiments at a facility producing clean, intense K^+ beams will unambiguously resolve such questions. It is therefore encouraging to note the announcement of a detailed design study for a kaon factory at TRIUMF Labs in Vancouver, made by the governments of Canada and British Columbia on 21 July. Among the features at such a facility would be a K^+ beam of intensity and purity orders of magnitude better than previously available.

The concept of nucleon swelling is controversial. Dunne and one of us (A.W.T.) have challenged, in the context of the data taken by the EMC, the consistency of interpreting dynamical rescaling as showing a significant increase in nucleon size⁸. Quite stringent limits have been placed on possible size changes by studying inelastic electron scattering⁹. Nor is the treatment of nucleon size in terms of vector mesons universally accepted. An alternative view is that in regions of momentum transfer probed by electron and K^+ scattering one should directly use a quark model¹⁰. One of the most fundamental questions in nuclear physics is whether or not these two pictures are equivalent. A recent calculation of the binding energy and density of nuclear matter in an explicit quark model suggests not. Guichon¹¹ found only a 2 per cent increase rather than the 10 per cent of Brown *et al.*⁵.

The controversy over the best description of nuclear physics at higher density is a sign of a healthy field. The existing paradigm of vector meson exchange may be reinforced or overthrown. The work of Brown *et al.* shows that experiments with intense, clean beams of K^+ mesons have an important role to play. □

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A.W. Thomas is in the Department of Physics and Mathematical Physics, The University of Adelaide, GPO Box 498, Adelaide, South Australia 5001 and J.E. Thomas is in the School of Physical Sciences, South Australian College of Advanced Education, Adelaide, South Australia 5000, Australia.

Daedalus

Deep green power

THE greenhouse effect is self-accelerating. The hotter the climate, the more power will be demanded for air conditioning, and the more carbon dioxide from fossil-fuel power stations will be poured into the atmosphere to heat up the greenhouse. Daedalus points out that carbon dioxide is readily soluble in water, especially under pressure. Water is also an ideal medium for dissipating the other waste product of power generation, low-grade heat. Because a great deal of fossil fuel, in the form of oil, natural gas and possibly beds of crystalline oceanic methane hydrate, is also located under water, Daedalus now puts forward the obvious answer. He is designing a submarine power station.

Many of the design problems have already been solved by the offshore oil rig and marine gas-turbine industries. But two fundamental problems need attention: how to get the air down to burn the deep-sea fuel; and how to get the resulting electricity ashore.

A big gill to extract oxygen from the water itself is probably impractical. Air will have to be drawn from the surface down a bulky pipe, preferably fitted with a sequence of compressors to balance the pressures inside and outside the pipe as it descends into the high-pressure depths. It will then be mixed with fuel and burnt in the gas turbine; the exhaust gas will simply be released into the deep, where its carbon dioxide will dissolve.

To get the electricity ashore, Daedalus boldly proposes to use the conductivity of sea water itself. The output winding of his unit will simply be a plastic tube full of sea water, with one end opened to the ocean. The pipe would be laid all the way to shore, where it would form the primary winding of the distribution transformer. Its other end would return to the ocean, completing the watery circuit. Thus the a.c. from the alternator would be carried between ocean and shore transformers by a complete loop of sea water. No electrodes would be needed, and no power would be wasted in electrolytic effects. The diameter of the current-carrying pipe could easily be made large enough to compensate for the relatively low conductivity of sea water.

Daedalus will give his power station neutral buoyancy, matching the neutral buoyancy of its plastic-pipe 'umbilical'. Thus it will be mobile. When one natural-gas borehole gives out, it will be able to move on to the next. It will also be able to 'browse' continuously over an ocean-floor field of methane hydrate, using its waste heat to disengage the weakly-bound methane. With no environmental impact at all, it will be the ultimately virtuous source of power.

David Jones

Was Taung human or an ape?

SIR—Conroy and Vannier, who concluded that the Taung fossil hominid had an ape-like pattern of tooth development and emergence on the basis of computerized tomography (CT) scans¹, have recently stuck to their view² in spite of evidence that demonstrates their error³. What CT really seems to show is that the Taung dentition developed according to a human pattern, but that the child may be younger than the 5–7-year old postulated on previously available evidence⁴. The dental developmental sequences which they claim are diagnostic of ape status in this specimen actually only separate American human juveniles from ape juveniles.

This is exemplified when non-American samples are examined, as Mann⁵ noted. Conroy and Vannier question² the existence of a Taung-like human specimen³, published by Mann, although both the excavation report and associated, but unphotographed, jaw fragments demonstrate its singular identity. Although it is not the "best example" of an ape pattern in this collection, it is one in which the dentition could be easily removed for clear photographic inspection, and is only one of a number of specimens sharing this pattern. Microscopy reveals no evidence of eruption of the lower incisor teeth whereas there clearly is a recently erupted M1; therefore it is more similar to what has been published as an ape-like pattern of dental development⁵ than Taung. Either simple comparisons of individuals with this reference⁵ is invalid or recent humans grew like apes.

Also, the second molar–canine dental sequence said to be diagnostically ape-like in Taung, and found in other fossils such as MLD (Makapansgat) 2 as well as the WT 15000 *Homo erectus* maxilla, is said to occur in living humans only one per cent of the time or less⁶. However this is a figure derived not from 'humans' but Americans. Examination of even a small sample (seven) of Near Eastern archaeological juvenile specimens with one of these two teeth just erupted, shows that the canine is earlier or simultaneous in five and the molar earlier in two.

A similar problem mars the claim that the timelag between first molar and central incisor eruption and development is diagnostically ape-like in Taung. If Taung followed a human pattern of dental development, the rootless condition of the incisors would not necessarily be unexpected for humans. The assertion that eruption of human I1 and M1 are simultaneous and, by implication, that these teeth should be in the same relative state of calcification¹, is a primary criterion in the claim of an ape-like pattern of dental development in Taung^{1,6}. Yet, a survey of 20 human samples world-wide shows the

range of mean incisor eruption delays behind first molar eruptions to be 0.3–1.9 years for the maxillary teeth. Although the lag is again minimal for American samples, it exceeds 1.5 years for almost one quarter of the samples and the mean difference between incisor and molar eruptions is 1.1 years. Thus the calcification status observed in Taung does not necessitate interpretation of an ape eruption sequence, as Conroy and Vannier claim¹. First molar eruption associated with a just-completed central incisor crown must be found within all human populations and must be the normal condition in many of them, based on these eruption data. These features of the Taung specimen, paralleled by a number of archaeological specimens, cannot distinguish between humans and apes. Neither Mann's data³ nor ours deny that apes and humans can be distinguished, only that they cannot be sorted on such a limited basis. So, neither the tooth development nor the implied emergence data reveal Taung as dentally like a 3.3-year-old ape.

Finally, we emphasize that the original primary criterion for ape-like status was its dental development pattern¹. Issues not relating to dental calcification and pattern, for example morphological features which they emphasised subsequently², tell us what most of us knew: Taung is not a modern human. These features cannot override or deny the essentially human (if un-American) pattern of the now clarified Taung dentition.

We can conclude from the new CT data that Taung is younger than previously supposed according to a human emergence pattern. An age of about 4.5–5.5 years, at the low end of the human range of population means for first molars like Taung's, would account for the dental condition of this australopithecine child. It is an earlier age at death, rather than the reflection of an ape-like eruption pattern, that best fits the CT observation on Taung.

MILFORD H. WOPOFF

Department of Anthropology,
University of Michigan,
Ann Arbor, Michigan 48109, USA

JANET M. MONGE

Physical Anthropology Section,
University Museum,

MICHELLE LAMPL

School of Medicine,
University of Pennsylvania,
Philadelphia, Pennsylvania 19104, USA

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Ozone destruction by algae in the Arctic atmosphere

SIR—Barrie *et al.* have shown¹ that ozone destruction occurs in the Arctic atmosphere during March and April of each year, as the Sun rises. They show that this phenomenon is linked to the photochemistry of bromoform formed biologically in the Arctic Ocean, and suggest that the production of bromoform is due to the decay of marine red benthic algae². But it is well documented³ that temperate marine macro-algae (such as the brown seaweed *Ascophyllum nodosum*) produce volatile organic compounds such as bromoform in substantial amounts (~10⁴ tons per year). This is of the same order of magnitude as the production by industry.

Maximal release of polybromomethanes by the two brown algae tested (*A. nodosum* and *Fucus vesiculosus*) was observed in October and November³. We have recently identified a group of novel vanadium-containing bromoperoxidases in brown seaweeds⁴ that produce free hypobromous acid (HOBr) and bromine (Br₂) in solution⁵. We assume that the bromoperoxidases are involved in the biosynthesis of these volatile halogenated metabolites by bromination of an unidentified nucleophilic acceptor in the algae. Although we have not yet carried out systematic studies, the presence of these vanadium enzymes in brown seaweeds shows a seasonal variation: maximal enzymic activity is found during the winter and spring, after which a rapid decrease is observed in the summer.

I thus propose that these vanadium enzymes are responsible for the production of bromoform and other halogenated compounds, and that this enzymic activity is linked to the observed ozone destruction. It would be of great interest to determine whether algal species present in the Arctic Ocean also contain enzymes involved in the biosynthesis of these halometabolites.

RON WEVER

Laboratory of Biochemistry,
University of Amsterdam,
PO Box 20151,
1018 TV Amsterdam,
The Netherlands

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Further analysis of multiexponential functions

SIR—One year after the publication of our paper in *Nature*¹, Tang and Norris published a scientific correspondence² concerning our work. It seems necessary

to me (Pierre Claverie died a few months ago) to address several of their comments on our approach.

An essential feature of our method is to combine together two tools, namely a functional transform and Padé approximants, to identify a given class of functions. In the paper we were concerned with the general class of complex exponential functions but we pointed out the generality of the method. A discussion of the two ingredients separately — and for their own sake — can thus be misleading. In fact, the only reference in the letter to the connection between the two tools reveals a severe misinterpretation of our approach.

The authors compare 'their' z -transform to 'our' Laplace transform. In our paper we stated explicitly that the so-called Prony's method can be reformulated as a Padé/ z -transform method, albeit with a severe constraint. We acknowledged a debt to the work of Weiss and McDonough¹ in making this connection.

Our approach is of course applicable to discrete experimental data, so the comment "as most experimental data are recorded as discrete points the z -transform seems to be more appropriate" is obscure to me. To identify exponential components we have used both the Laplace transform and the z -transform. We have resorted to the z -transform variant allowed by our approach in cases characterized by 'sparse data' when "the time constants are of the order of magnitude of the sampling step"¹. Introducing p in the z -transform through $z = \exp(p\Delta t)$ we see trivially that the z -transform is a numerical approximation (Riemann sum) to the Laplace transform which becomes exact in the limit $\Delta t \rightarrow 0$. The poles of the Padé approximants are now $z_i = \exp(\mu_i \Delta t)$ instead of $p_i = \mu_i$, where the μ_i are the exponents of the exponentials. In our approach the two transforms are thus complementary: we use the z -transform when the sampling step is of the same order as the time constants. The Laplace transform becomes effective at the other extreme (small Δt), in which case the poles of the Padé approximants become difficult to discriminate when the z -transform is used as they all tend towards the value 1. Also, by definition, the z -transform can deal only with regularly sampled points while such a constraint does not apply to the Laplace transform. Indeed, we have developed such a version of our program, which proves to be very useful in experimental situations with time constants spanning several orders of magnitude.

The Taylor series coefficients of the functional transform are the input to a 'black-box' which calculates the coefficients of the Padé approximants which, in the cases of the exponential components, reconstitute the analytic expression of the Laplace or z -transform. In a major criti-

cism the authors state that we use only $2M$ experimental points (M being the length of their LP filter). The parameter restricted in our approach is not the number of experimental data points but the number of Taylor series coefficients. Each term of the Taylor series is calculated using all the available experimental values. The only case in which each term coincides with a single experimental value is the case of the z -transform with $z = \infty$ (which is again equivalent to the Prony method and is precisely what we avoid). In our method the finite value of z allows each Taylor coefficient to convey information about the whole curve. Therefore, this should represent the best direct method (that is, with no linear or non-linear least-squares step) of component identification.

The limited number of Taylor coefficients we use (usually ~ 24 for Padé approximants up to order 12) is also advantageous numerically compared to the usual $M=0.75N$ linear prediction coefficients (N being the number of experimental points) used in the LP approaches⁵ mentioned in the correspondence. Thus in ref. 5 calculation times of one hour are reported for 1,024 points using the LPSVD method on a minicomputer. Our program involved calculation times of 25 s for 1,890 points on an IBM XT compatible microcomputer. We are not limited by matrix size as the number of Taylor coefficients in our method does not depend on the number of experimental points. In addition we consider only polynomials up to order 12 while in the LPSVD method "the order of the polynomial can be as high as 750" (ref. 5).

I am happy that the authors acknowledge the importance of the choice of the parameter p (or z). It is suggested that this could be performed within LP with a "slight modification of the equations". I am unable to detect in the cited papers any mention of such a choice or of any basis on which it should be made. (After the publication of their correspondence Tang and Norris wrote a paper² describing the 'LP-ZOOM' method, in which they introduced for the first time the equivalent of a free choice for the parameter z in the z -transform.) Finally, in our approach the free choice of the parameter p allows one to deal with truncations: high values of p have the effect of smoothing off such cuts.

Longman's algorithm⁶ is nothing other than a (very) tricky numerical tool that allows one to manage the calculations in the 'black-box' in a very efficient way. To solve the linear system equations corresponding to each Padé approximant we may use simple determinants. Longman's algorithm allows one to calculate the Padé coefficients up to a prescribed order using a recurrent procedure which reduces the calculation times significantly. Accordingly the Table in the correspondence, in which the authors compare the LPQRD

and Longman methods, relates only to the numerical performances of the original Prony procedure.

I hope that a positive result of this controversy will be to highlight the necessity of a conceptual synthesis in this field of great importance to many experimentalists. From the user's point of view, I think that it is also very important that objective comparisons are performed. I propose that a set of standard tests should be devised to which all available and new methods should comply.

E. YERAMIAN

Unité de Physicochimie des
macromolécules biologiques,
Institut Pasteur,
28 rue du Dr Roux,
75724 Paris Cedex 15,
France

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Antifreeze polypeptides come out of the cold

SIR—Yang *et al.*¹ have recently proposed an elegant mechanism for the preferential alignment of antifreeze polypeptides (AFP) at the surface of growing ice crystals and the subsequent modification in crystal habit. They postulate that dipole interactions between the α -helical polypeptide molecules and water molecules in the crystal lattice are maximized for anti-parallel alignment along the $\langle 221 \rangle$ crystallographic direction (Yang *et al.* have stated that in ref. 1 $\langle 112 \rangle$ should read $\langle 221 \rangle$) and this results in the stabilization of the prism faces to further growth. Here we highlight several points that make this hypothesis untenable and suggest a much simpler explanation for the crystal habit modification observed in the presence of AFP.

First, the mechanism does not account for the stabilization of the prism faces. Alignment of the AFP along $\langle 221 \rangle$ can occur within a multitude of different crystal planes that all lie parallel to the $\langle 221 \rangle$ zone vector. Thus, the proposed preferential adsorption of AFP on the prism faces cannot be explained by a unique helical alignment along the $\langle 221 \rangle$ direction. Second, it seems implausible that the ordering of water dipoles by the α -helices at the crystal surface will have a significant influence on the relative rates of growth of different faces. Although the addition of water molecules to an ordered face will be less entropically favourable, the alignment of dipoles only exists in the presence of the field imposed by adsorbed AFP molecules; as addition of a further water molecule to the crystal lattice requires the transient desorption of a

bound AFP, the surface dipoles will become randomized during incremental growth. Third, the mechanism provides no explanation for the subsequent alignment of the helices parallel to the *c* axis. Indeed, it is not clear why this should be an important requirement. Helix-helix interactions could take place whilst maintaining the proposed preferential $\langle 221 \rangle$ orientation.

The observed habit modification can be explained as follows. In the absence of additives, crystal growth parallel to the *c* axis is slow because the corresponding basal face has the lowest surface energy; this is due in part to the relatively large number of periodic bond chains that lie parallel to the puckered sheets of this surface. Adsorption of polar additives will be unfavoured on the basal face simply in terms of energetic considerations, and will occur on faces of higher surface energy such as the prism faces. There is therefore no need to invoke either epitaxial² or dipolar¹ arguments for the low adsorption of AFP on the basal face. Furthermore, these mechanisms would result in the specific expression of a unique set of higher energy planes in the crystal morphology. This is not observed: for example, a range of curved faces of variable index $\{1011\}$ are reported³. The elongation along the *c* axis is a reflection of the increased stabilization of the high surface energy planes perpendicular to this direction. In this respect, the discussion in the paper by Yang *et al.* of amphiphilicity and torsional freedom is pertinent because the asymmetrical distribution of charge along the AFP helix generates strong adsorption and a hydrophobic crystal interface which inhibits the further addition of water molecules to the surface.

Finally, we note that the function of AFP is to inhibit ice formation, and not to modify crystal habit as described above. Inhibition involves the destabilization of ice nuclei which may be of only nanometre dimension; these clusters are unlikely to exhibit the well defined crystal structure and faces of microscopic crystals, with the consequence that epitaxial and dipolar interactions with AFP cannot be established, although surface energy considerations remain applicable. A close correspondence between the length of an AFP molecule (5 nanometres) and the critical cluster size for ice nucleation, combined with the asymmetrical and extended charge distribution and hydrophobicity of the α -helix, are probable factors that should account for the effective disruption of ice nuclei forming in the plasma of polar fishes.

S. MANN
B.R. HEYWOOD

School of Chemistry,
University of Bath,
Bath BA2 7AY, UK

YANG ET AL. REPLY—The crux of the “much simpler explanation” proposed by Mann and Heywood is their assertion that “adsorption of polar additives will be unfavoured on the basal face simply in terms of energetic considerations, and will occur on faces of higher surface energy such as the prism faces”. Their statement is inconsistent with the properties of hexagonal ice. The basal and prism faces are the ones with the lowest energy⁴, and the fact that their surface energies are probably similar is indicated by the temperature dependence of the morphology of ice crystallized from supersaturated water vapour⁵; moreover, both faces contain three intraplane hydrogen bonds per water molecule, the density being somewhat higher on prism faces⁴. Surface energy does not provide an unambiguous explanation for the binding of AFP to prism faces. On the contrary, AFP should not bind to either the basal or prism faces from surface energy considerations alone.

The morphology of crystals can be altered by specially designed substrates which adsorb only to specific faces when introduced into the mother liquor (see ref. 6 for review), and can stereochemically inhibit growth normal to the adsorbing face. According to our mechanism, as part of the tailoring of AFP for its function, the relative orientations of the ice dipoles and the prism faces direct the binding of the protein. Although the mechanism allows the protein to bind to any plane containing the $\langle 221 \rangle$ vectors, the most frequently encountered of these ought to be the prism face because it has the lowest surface energy. At this time we are unable to explain the reported development of a range of curved faces of variable index $[101x]$ when a hexagonal ice crystal is transferred into a solution containing a mixture of four different antifreeze glycoproteins³.

The tendency of the helices to align themselves more nearly along the *c* directions with increasing concentration of AFP reduces the unfavourable contacts between helices arising from the hexagonal symmetry. We agree that the realignment may not be an important requirement of the mechanism at low protein concentrations, but it seems reasonable to expect protein-protein interactions to influence the steric arrangement as the protein-to-water ratio increases in the complex.

With reference to the comments on the significance of the ordering of the ice dipoles by the helices on the surfaces, it is obvious that the reduction in entropy, both at the surface and at regions in bulk of the ice crystal near the surface, will make these sites less favourable for further growth⁷. The argument posed about protein desorption seems obscure, if not irrelevant. At equilibrium the prism faces will always contain adsorbed AFP, despite

dynamic exchange with protein molecules in the bulk solution. Furthermore, growth along the *c* axis may occur independently of the protein molecules bound on the prism faces when the diameter of the exposed basal plane's surface is large enough. As the diameter shrinks, the entropy reduction in the bulk ice may become large enough to halt growth in the *c* direction also.

Finally, the relevance of the proposed mechanism to the inhibition of the development of ice crystals from nuclei is questioned. Nuclei larger than the critical size (~ 400 Å; ref. 2) grow spontaneously. Accordingly, as they are likely to possess well defined faces, the mechanism applies directly.

D. S. C. YANG
M. SAX

Biocrystallography Laboratory,
VA Medical Center,
Pittsburgh 15240, USA

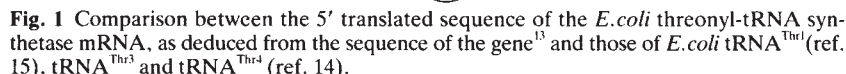
A. CHAKRABARTY
C. L. HEW

Departments of Biochemistry and Clinical
Biochemistry,
University of Toronto,
Toronto, Canada M5G 1L5

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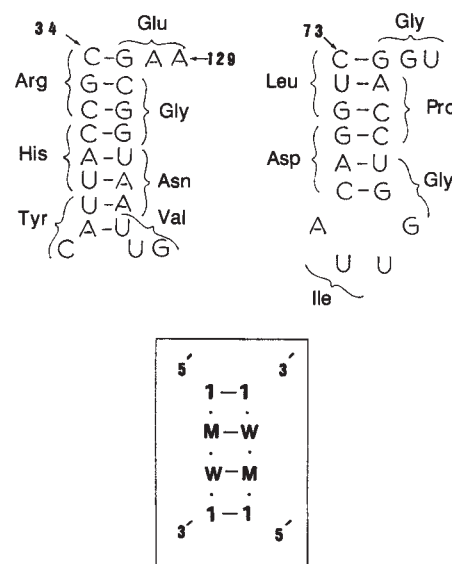
Evolution of new tRNA-synthetase classes

SIR—Molecular regulation involves a variety of mechanisms whose role in the history of an interactive system remains unclear. Despite extensive variation in primary structure, aminoacyl-tRNA synthetases do catalyse the same basic reactions, and use similar mechanisms to control their biosynthesis: transcriptional autocontrol¹, attenuation of transcription² and autocontrol at the post-transcriptional level *in vitro*³ and *in vivo*⁴. *In vivo* autocontrol could be achieved by a translated region of the *Escherichia coli* threonyl-tRNA synthetase (*thrS*) mRNA folding into a cloverleaf structure that would be bound by the synthetase, preventing ribosome elongation⁵. Genetic analysis of the regulatory region using *thrS-lac* hybrids showed, however, that the translational operator was located within 30 base pairs (bp) in front of the Shine-Dalgarno sequence, and that there was some nucleotide homology with tRNA^{thr} isoacceptors, particularly in the anticodon loop and stem⁵. In the light of the suggestion by Ho⁶ that self-splicing of primitive tRNA to yield a ribozyme would be coupled with aminoacylation, I



A curious feature of the 5' translated mRNA sequence of the *E. coli* threonyl-tRNA synthetase, shown in Fig. 1, is that it displays extensive homology with the *E. coli* tRNA^{Thr} isoacceptors: 17 base pairs and 19 nucleotides out of the 94 which form the entire structure are located in similar positions. The R-N-Y codon pattern represents 43% of the sequence and may reflect the mRNA archetype as suggested by Eigen and Schuster⁷. Analysis of the base pairings of the codon nucleotides within the equivalent of the aminoacid and anticodon stems shows that the first base of each codon faces the first base of the opposite codon (Fig. 2). The arrangement could maintain the importance of the middle nucleotide at the expense of the wobble one; it is interesting that some

The RNA structure of a mRNA coding for a part of an aminoacyl-tRNA synthetase resembles that of a tRNA and may therefore have yielded a polypeptide capable of proof-reading the tRNA, yielding a highly accurate system coupled to the aminoacylation reaction. The features of the most efficient system might later have been encoded as DNA. That would account for the diversity of tRNA-synthetase-specific interactions, exemplified by the recent discovery of nucleotides in tRNA governing their identity^{11,12}. It is worth noting that the third base pair in the amino-acid stem of tRNA^{Thr1} and tRNA^{Thr3}



Experiments with the basic elements described here may help in the exploration of roots of biocatalysis at the evolutionary level.

Inserm U.298,
Centre Hospitalier Régional Universitaire,
49033 Angers Cedex, France

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Tales of future past

Henry Gee

The New Dinosaurs: An Alternative Evolution. By Dougal Dixon. Grafton, London/Salem House, Topsfield, Massachusetts: 1988. Pp.120. £12.95, \$19.95.

IF YOU are going to tell a lie, make it a big one. Then enough people will believe it for it to be accepted as fact. This philosophy might be formalized as Münchhausen's Law of the Tall Story, and its incidence in science is on the increase. A notable example is the idea that the history of life took an unscheduled detour when a large meteorite hit the Earth at the end of the Cretaceous, about 65 million years ago. The dinosaurs — the dominant land vertebrates — became extinct as a direct consequence, along with the ammonites, a group of marine cephalopod molluscs, and many other taxa. After the collision, the world was left empty for mammals and birds to colonize, and the rest is biological history.

As evidence in support of the Münchhausen Meteorite becomes more convincing, the idea that life on Earth was beset by a catastrophe at the end of the Cretaceous is becoming a part of the palaeontological mainstream. So much so that it is safe to ask "What if the asteroid missed, and life carried on as normal?", without being thought reactionary. In *The New Dinosaurs* Dougal Dixon starts from the premise that the asteroid did indeed miss and imagines the animals that might have existed now but for the rude extraterrestrial interruption. In Dixon's scheme the dinosaurs are still dominant, but they have adapted and diversified to fill the Tertiary and Quaternary ecosystems. Dinosaur versions of deer, bovids, elephants and rhinos graze the grasslands (a habitat largely unknown before the Tertiary and only limited in extent before the Pliocene). Some of the grazers are not dinosaurs but cursorial, ground-living pterodactyls. Trees around the world are full of monkey-, squirrel- and bird-like coelurosaurs. Some of the ecological parallels are almost cheekily close; there is a Neotropical manatee analogue as well as an Oriental version of the dugong, both hypsilophodonts. There is a dinosaur giant panda that lives solely on bamboo, and a coelurosaur woodpecker that chisels its way into trees.

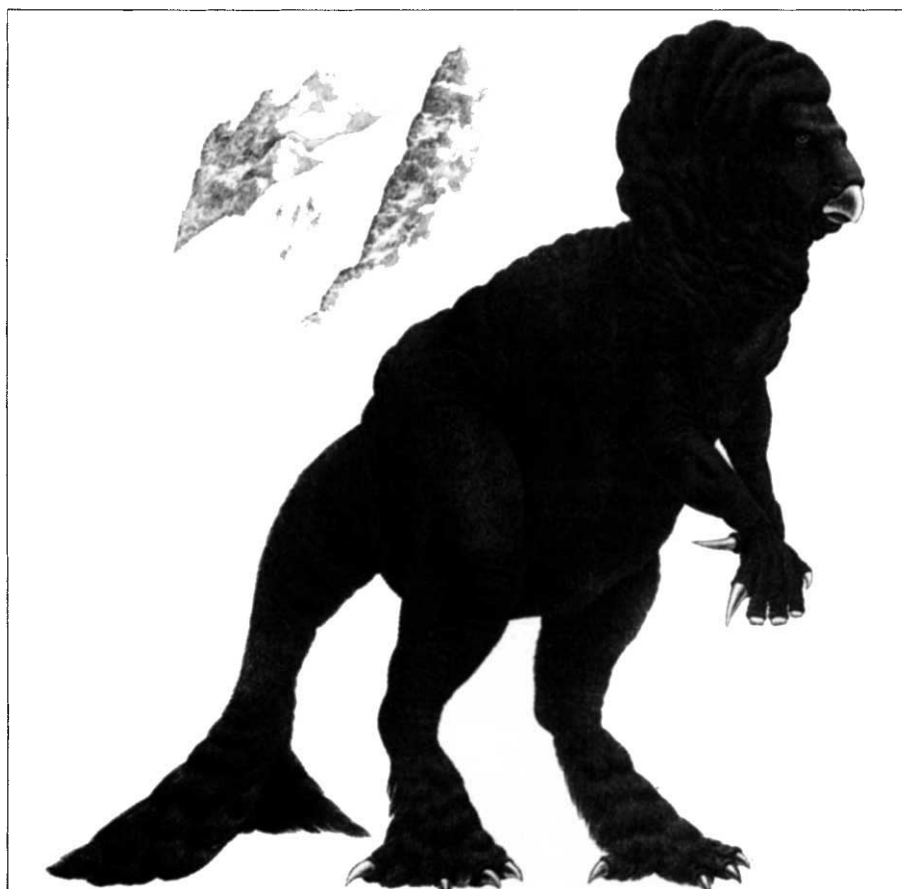
At first sight the book appears to be merely a colourful diversion. But Dixon has taken great pains to play the game by the rules of evolution, and in a few concise pages shows how organismal diversity is constrained by ancestry and development, geographical accident and the availability of ecological niches. From there he moves on, in the main body of the book, to

discuss each of the zoogeographical realms in turn, bringing all of the biological and environmental factors to bear in conjecturing the course of dinosaur evolution. In *On Fairy Stories*, J.R.R. Tolkien said that for a fantasy to be believable, it must be internally self-consistent. Dixon's book, then, owes more to Tolkien than to Münchhausen in that the rules governing his fantasy are clearly stated and meticulously followed. Through his adherence to darwinian rules he has found an ideal vehicle for presenting basic ideas about evolutionary diversity and the principles that underlie biogeography.

So, underneath the flummery, here is a book that is intellectually satisfying. Yet it is also hugely entertaining; as a celebration of evolutionary possibility, it is nothing short of riotous. The presentation takes a

form rather like that of an illuminated mediaeval bestiary, and many of the names Dixon has chosen for his creations owe more to Edward Lear than Linnaeus. The "harridan" is a condor-like pterosaur; the "coconut grab" is an ammonite that comes ashore on tropical islands; and nectaries of tropical orchids are raided by the tiny "gimp". The table manners of the "gourmand", however, are far from delicate — the beast in question is a horrific 60-foot scavenging tyrannosaur that swallows entire carcasses whole.

In format, the book takes after its predecessor, *After Man*, an illustrated account of how life on Earth would look 50 million years after the extinction of mankind, although the emphasis here is more on biogeography than on natural selection. Dixon acknowledges that the idea behind *The New Dinosaurs* is not new, and presents a potted critique of books and films on the same theme. Here he addresses the question of intelligence, to rebut criticisms that 200 million years of evolution would surely have produced a dinosaur with an intelligence on a par with that of humanity. Dixon's excuse for not inventing an equivalent of (say) an animal Charles Darwin is that such intelligence



Acclimatized to cold — the Balaclav (*Nivesaurus yetiforme*) a dinosaur which in Dougal Dixon's scheme evolved a shaggy coat and deep fat layers to insulate it from the rigours of alpine environments, and a beak and nails adapted for subsisting on lichens, moss and alpine plants. The creature "lives in small family groups in the highest mountains [and] can often be seen trekking across snowfields and glaciers from one lichenous rock or mossy hollow to another".

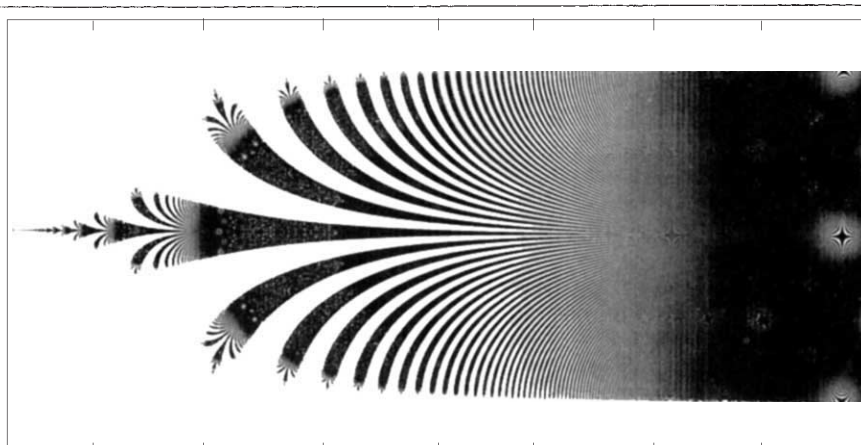
would have resembled "animal cunning" rather than "the kind of intuitive reasoning that we associate anthropomorphically with the term" (p. 112). This assertion is lame; having explored the consequences of the initial premise as far as he does, it is a pity that Dixon baulks at the final hurdle.

Another flaw of the book is that reproductive physiology is treated rather vaguely, at least from my own (mammalian) perspective. But such criticism could be misplaced. Our own self-esteem as mammals demands that much — perhaps too much — importance is placed on higher mammalian specializations in the great scheme of things. Dixon's book is well in tune with current trends in evolutionary palaeontology, in which mammals owe their place as much to historical accident as to any inherent design superiority over dinosaurs.

Only one mammal is described in detail, a rat-like aquatic placental, which is probably not very different from the earliest eutherians. Monotremes and marsupials do exist in Dixon's scheme, but apart from a bald statement of the fact in a figure, they are mentioned no more; all the familiar niches for large marsupials in Australasia are occupied by dinosaurs. Birds, likewise, get little attention. Although they are referred to frequently as successful aerial specialists along with the pterosaurs, only two species are mentioned in particular. Both are flightless. The constant bracketing of birds and pterosaurs would, I think, have been better accompanied by more consideration of how the ecologies of birds and pterosaurs differ and of why excessive competition between them is thereby avoided. But as with mammals, the birds should be seen as a reptilian offshoot rather than a group deserving class status. If the asteroid missed, the problems of reptile paraphyly would never have arisen, because birds and mammals would have had the same rank as any other amniote subgroup; and cladists would have had much less to talk about.

The final irony is that Dixon may not be very far off the mark, because many of his creations could well have trodden the Earth, or flown in the skies above it, during the Mesozoic. The fossil record is so poor that we can only know of a tiny fraction of the number of dinosaur species that existed. Animals living in most habitats, especially in areas of net erosion such as mountains, fossilize poorly. Dixon's "balaclav" (an alpine, yeti-like hypsilophodont, shown on the previous page) or "mountain leaper" (a fluffy warm-blooded coelurosaur that darts from pinnacle to pinnacle) could very well have existed outside the two-dimensional confines of his book. □

Henry Gee is on the editorial staff of Nature.



Infinite wisdom — fractal patterns of surprising complexity can be produced using simple iteration schemes on a microcomputer. Here the Julia set of the function $\frac{1}{c^2}$ is plotted in the complex plane. Points are coloured black if their path, or 'orbit', through the complex plane, determined by the iteration process $Z_{n+1} = \frac{1}{c} e^{Z_n}$ eventually escapes to infinity. The orbits of white points, however, all tend to the fixed point 1. The picture is taken from *The Science of Fractal Images* edited by Heinz-Otto Peitgen and Dietmar Saupe. Publisher is Springer-Verlag, price DM68, £23, \$34.

Founding father

Robert Temple

Theophrastean Studies: On Natural Science, Physics and Metaphysics, Ethics, Religion and Rhetoric. Edited by William W. Fortenbaugh and Robert W. Sharples. Transaction Books, New Brunswick, New Jersey/Clio Press, Oxford: 1988. Pp. 348. \$49.95, £36.75.

THEOPHRASTUS of Eresus in Lesbos was a younger friend of Aristotle, and they were students at Plato's Academy together. After Plato's death, Theophrastus became Aristotle's chief assistant in a massive programme to study the whole of nature, Aristotle taking animals as his subject and Theophrastus taking plants. They thus became the 'fathers' of zoology and botany, respectively, and their works followed a common plan. As R.W. Sharples puts it, they both saw "the natural world as *in principle* the object of a single, unified enquiry". After Aristotle's death in 323 BC, Theophrastus became the head of their school, the Lyceum, at Athens, where he had over 2,000 students.

Lists of his works by later librarians at Alexandria give more than 270 titles. Over 1,000 fragments of these have been gathered, edited and translated by the International Theophrastus Project and will be published within the next few years. The contributors to this volume are leading members of the project, and this is the volume intended to act as an introduction to the forthcoming fragments. (A text and translation of Theophrastus's *Meteorology* discovered in a Maharajah's library in India by Hans Daiber will appear as a separate volume.)

The book is not fully accessible to

readers without some knowledge of Greek philosophy and philosophical terminology. But it has an importance far exceeding its forbidding presentation. The attention given to Theophrastus over the past decade, reaching its culmination here, makes possible for the first time a proper understanding of the methods, aims and achievements of Aristotle in his scientific works. In their biological writings, both Aristotle and Theophrastus are now seen to have avoided trying to construct a taxonomy. Their approach to understanding nature was entirely different, their joint efforts being directed towards discovering natural causes by a method derived from Aristotle's theories of logic, and which sought to elucidate and identify differences of distinctive features in the widest possible general classes. Their works were neither natural histories nor taxonomic enquiries, and until today a true appreciation of their intent has been entirely lacking. Historians of science will have to grapple with the bizarre implications of these findings, for we have here nothing less than the revelation that Western science emerged originally from systematic concepts and methodologies wholly different than had been thought until now.

One of the best contributions is by Alan Gotthelf, co-editor with James Lennox of another seminal work on the subject, *Philosophical Issues in Aristotle's Biology* (Cambridge University Press, 1987). *Theophrastean Studies* also portrays Theophrastus as a founder of ecology, student of pharmacology and historian of religion, and together the two books are of the greatest possible importance for those studying the roots of Western science. □

Robert Temple, Hill House, Sutton Mallet, Bridgwater, Somerset TA7 9AW, UK, is author of, among other books, *Conversations with Eternity, a comparative study of ancient proto-science* (Hutchinson, 1984).

Moving on the spot

Peter D. Moore

Plant Migration: The Dynamics of Geographic Patterning in Seed Plant Species.

By Jonathan D. Sauer. University of California Press: 1988. Pp.282. \$45, £26.95.

THERE are occasions when words fail us; one word which is quite inadequate when applied to the plant kingdom is 'migration'. It generates visions of weary herds of wildebeest or caribou, or of swallows on telephone wires, but in the plant world it has come to mean something quite different. Rather than the active mobility of the adult individual, one must think in terms of alterations in distributional boundaries resulting from the invasion and establishment of propagules on one side and the extinction of populations on the other. We need a new term for this process, which is also found in sessile animals such as barnacles and corals.

Sauer subtitles his book *The Dynamics of Geographic Patterning in Seed Plant Species*, which is not a particularly snappy alternative to *Plant Migration* but which does explain the author's interpretation of the term. His approach to the subject is unusual, for most authors of books on plant geography begin with general principles and then illustrate them by example. In contrast, Sauer contents himself with the documentation of numerous case histories upon which meaningful conclusions may be based. This method is commendable in theory, for the reverse process always involves selecting those examples which most effectively illustrate the principle, so if the principle is defective it nonetheless appears to be supported by the data. Sauer's method, however, can leave one lost in a maze of information with little trace of an underlying theme to guide one into the generalities.

The arrangement of the case histories falls into three parts — recent migrations (those which have been historically documented), prehistoric migrations (those revealed by the fossil record, both recent and ancient), and those that have been accompanied by evolutionary changes (mainly based on recent examples, such as crop mimicry by weeds and the colonization of mine spoil). Within these divisions, the examples are further classified on the basis of the nature of the invaded site (naturally disturbed, such as shorelines or volcanoes; stable, as in the invasion of established forest; and artificially disturbed, where human activity is involved) or, in the case of the fossil record, the subdivision is based on time considerations and on geographical area.

The case histories vary greatly in their

degree of detail. The surveys of weed invasions and coastal movements are perhaps the most informative and full, and will provide a most useful source. The fossil accounts are far from satisfactory and contain only the most superficial information on species boundary changes in time. The task, however, is formidable, and the carefully selected bibliography is a redeeming feature.

My main criticism of the book is the difficulty in finding answers to the most obvious questions. How fast did tree migration take place in the development of our current interglacial? What are the factors influencing the retreat of a distribution boundary rather than its advance? What is the role of climatic thresholds in plant geographical dynamics, and how

precisely do they operate via the physiology of the plant? Such questions are not directly addressed.

The book lacks general conclusions, except, perhaps, that the complexity and variability of plant migration in the real world defies generalization. My fear is that this compendium will be consulted mainly by those interested in compiling information to support their own theories of plant migration rather than those who, as the author seems to hope, will look first at the wealth and range of the data and then begin to formulate broad conclusions. □

Peter D. Moore is in the Division of Biosphere Sciences, King's College London, Campden Hill Road, London W8 7AH, UK

Pandora's black box: 'mosaic' thermodynamics

S. Roy Caplan

Thermodynamics and Control of Biological Free-energy Transduction. By Hans V. Westerhoff and Karel van Dam. Elsevier: 1987. Pp. 568. Dfl 450, \$200.

THE brightly-coloured motif on the cover of this elegantly printed and bound book is one of a series of charming illustrations which punctuate the text. It depicts a black box partly opened to reveal a *mélange* of densely packed mathematically-shaped articles, some transparent, the majority not.

Although intended to represent the subject, the motif is a remarkably accurate representation of the book itself. Westerhoff and van Dam have set themselves a task of Herculean proportions: to describe how the fundamentals of physics and chemistry apply to biology. As they point out, some 35 per cent of their text provides biologically-orientated background material, including statistical mechanics, equilibrium thermodynamics, nonequilibrium thermodynamics, metabolic control theory, cell biology and optimization theory. Another 25 per cent is devoted to a review of the contemporary literature of nonequilibrium thermodynamics in biology, emphasizing the approaches taken by the authors and their colleagues. The remainder deals essentially with unpublished studies of the control analysis of biological free-energy transduction.

All this (and more) is encompassed in four chapters, the first three of which are devoted in turn to the basis for, the core of and the applications of what is termed 'mosaic' nonequilibrium thermodynamics. This discipline takes in a formid-

able number of topics. A random sampling of these yields the master equation approach to nonequilibrium systems, the Langevin approach, the diagram method in kinetics, the stability of metabolic states, responses to fluctuations, Euler's theorem (and the derivation of the summation theorems of metabolic control theory), flow-force relationships for elementary processes close to and far from equilibrium, ion transport in bacteriorhodopsin liposomes, mitochondrial oxidative phosphorylation and microbial growth. Even this list constitutes only some five per cent of the goodies to be found in Chapters 1–3. Chapter 4 is devoted entirely to metabolic control, including two practical examples: bacteriorhodopsin in liposomes and oxidative phosphorylation in mitochondria.

Wide ranging as all this is, in a book dedicated largely to nonequilibrium thermodynamics (especially one in the distinguished Dutch tradition) it is surprising to find no mention of the work of Staverman and his followers, at least for the sake of the novitiates. Ion transport is considered in a limited context (bacteriorhodopsin liposomes); water transport is touched on only marginally. Arguably these matters may be peripheral to the authors' considerations, but even more surprising, in view of the methodology of the book, is that the detailed studies of mosaic systems carried out some 30 years ago by Kedem and Katchalsky are ignored. In those studies it was demonstrated that an array of different membrane elements can exhibit new properties not associated with the components of the array. This important general characteristic receives no comment here, although the approach is identical: the elemental flows are summed for the steady-state condition, yielding a complex matrix of conductance coefficients which may itself be thought of as resembling a mosaic. Unluckily Kedem and Katchalsky did not label their work 'mosaic' nonequilibrium

thermodynamics: for them it was just the everyday common or garden variety.

Despite these lacunae and numerous technical details which purists will dispute, Westerhoff and van Dam have presented bioenergeticists with an extraordinarily broad, if eclectic, compendium of well-integrated theoretical and experimental information, together with an alphabetical bibliography of nearly 700 references. The accounts of current work may become obsolete, but for the time being they are undoubtedly worthwhile contributions. Control theory has lacked a definitive textbook presentation, and although the sceptics will probably not be convinced by this one, it is comprehensive and gives them plenty to think about. All this may make the book attractive for libraries, but the individual scholar will ponder carefully before investing in it. His best bet is to borrow a copy, classify himself according to the eight categories of reader defined by the authors, and then to try navigating the route designated for him. Much of the mathematics and associated terminology will prove heavy going for biologists, although those with a physical bent should be stimulated to turn to the original sources for more complete treatments of topics like the diagram method.

The book is written in a lively style, is adequately indexed and has a helpful glossary of symbols, but would have benefited from thorough editing. It suffers from a surfeit of parentheses — for example on p.115 we find, "Noting that the (co)variance (matrix) of the equilibrium distribution is equal to (contains) the second derivatives of the (local) characteristic function . . .". This sentence also illustrates the level of mathematical expertise required. Many English-speaking readers will be disconcerted to find the pound sterling derogated to the role of a superscript, while the Gothic spelling of Gaussian looks decidedly odd. But these are forgivable shortcomings in an otherwise well-crafted product. □

S. Roy Caplan is a Professor in the Department of Membrane Research, The Weizmann Institute of Science, 76 100 Rehovot, Israel.

New in paperback

- *The Omega Point* by John Gribbin. Publisher is Corgi, price is £4.95, \$8.95. For review see *Nature* **330**, 294 (1987).
- *On Food and Cooking: The Science and Lore of the Kitchen*, edited by Harold McGee. Publisher is Collier Macmillan, price is \$16.95. Available in Britain from Unwin Hyman, £12.95. For review see *Nature* **314**, 701 (1985).
- *Biotechnology: The University-Industrial Complex* by Martin Kenney. Publisher is Yale University Press, price is \$14.95, £9.95. For review see *Nature* **325**, 669 (1987).
- *Who Goes First? The Story of Self-Experimentation in Medicine* by L. K. Altman. Publisher is Equation, price is £8.99. For review see *Nature* **330**, 429 (1987).

A shock to the system

Peter D. Marshall

The Great Tangshan Earthquake of 1976: An Anatomy of Disaster. Edited by Chen Yong *et al.* Pergamon: 1988. Pp.153. £20, \$36.

ON 28 July 1976, a huge earthquake, 7.8 on the Richter scale, occurred beneath the Chinese city of Tangshan. Because the focus of the earthquake was shallow, the intensities within the immediate vicinity of the epicentre were extremely high and Tangshan, a large industrial city, was totally destroyed. The final casualty toll was horrendous — 240,000 people died and over 700,000 were injured. Such a catastrophe warrants thorough analysis. But although numerous studies have indeed been made of the scientific and socio-economic aspects of the Tangshan earthquake, most of them have been published in Chinese. This slender volume summarizes, in English, the salient points contained in the Chinese reports. The authors have done an excellent job, and have produced a first-class book.

The failure to predict the Tangshan earthquake must have been a big disappointment for Chinese seismologists, who only 18 months earlier had distinguished themselves by successfully predicting a magnitude 7.3 earthquake in the nearby Haicheng region of north-east China. This failure, in an area identified by the Chinese as "an earthquake-hazardous zone" that had been designated "for special attention as far as prediction is concerned", is dealt with in detail in the book. Although in retrospect it is possible to recognize numerous precursive indications which occurred one to two days before the main shock, they were in fact too few and too late to give the scientists a chance of making any forecast. This highlights the difficulties of earthquake prediction; the large variety of triggering mechanisms is such that sometimes there will be precursory phenomena and sometimes not. As the authors point out, "by bluntly expecting the Tangshan case to duplicate the example of the Haicheng pattern, the prediction attempt was doomed at the very outset".

The text is divided into four chapters. Each of them can be read without reference to the others, which means that the book will be of easy use both to those in disaster relief agencies and to scientists. Chapter 1 describes the destruction of the socio-economic infrastructure and the civil engineering aspects, and Chapter 2 the human aspects and the rescue details. The other two chapters are more scientific in content, and are concerned with seis-

IMAGE
UNAVAILABLE
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REASONS

Living on the edge — the tractor of the Daodi Commune, Fengnan County, was a victim of the earthquake.

mology and earthquake prediction. Here, important questions are raised which will need to be resolved if the science of prediction is to be taken seriously.

All six of the authors are members of China's State Seismological Bureau. They were all on the disaster scene within hours of the main shock and were eyewitnesses to the devastation. They participated in the rescue work and in the reconstruction of Tangshan, and were obviously deeply affected by what they saw.

But this is not just a horror story; rather it is a very valuable chronicle of what actually happens during and after a major and damaging earthquake. Most particularly, there are many lessons to be learned from it on how to respond to the immediate needs of the victims — to be sure, few nations could have dealt with such a catastrophe as effectively and efficiently as the Chinese. □

Peter D. Marshall is a Principal Scientific Officer at the Ministry of Defence Seismology Unit, Blacknest, Brimpton, Reading RG7 4RS, UK.

Distinct dental development patterns in early fossil hominids

A. D. Beynon* & M. C. Dean†

* Department of Oral Biology, The Dental School, Framlington Place, Newcastle-upon-Tyne NE2 4BW, UK

† Department of Anatomy and Developmental Biology, University College London, Gower Street, London WC1E 6BT, UK

New studies on the jaws of hominids, based on incremental growth markings in teeth, can now provide an absolute timescale with which to calibrate dental developmental events such as tooth emergence. These new estimates of crown-formation times and the observed sequences of dental development are different in the hominids Australopithecus and Paranthropus. Early hominids evidently had shorter periods of dental development than modern humans and therefore a less prolonged infancy.

THE claim by Raymond Dart¹, that the juvenile skull from Taung was morphologically distinct from the great apes met with general disbelief from the scientific community of 1924. Gradually, however, with the discovery of more specimens, it became generally accepted that Dart's diagnosis of Taung as a hominid was correct. Since that time, descriptions of new hominid fossils have tended to stress their human-like features and thus emphasize the distinctions between early hominids and great apes. It was natural for this trend to extend to studies of hominid growth and development, so it was claimed that hominids shared an extended or prolonged period of growth and development with modern humans^{2,3}. The implications of an extended growth period are profound, and would include an increased parental investment⁴ with prolongation of the period of childhood dependency, and its consequent influence on 'human' behaviour, learning and culture³.

More recent studies on fossil hominid dentitions using incremental growth markings in teeth⁵⁻⁸ (visible both externally and internally), together with detailed studies on dental developmental stages^{9,10}, have reached a different conclusion. Periods of early hominid growth and development were apparently short, although there is still some debate over this conclusion¹¹⁻¹³. It is also becoming apparent from such studies that early hominids possessed unique patterns of dental development, differing not only from modern man and apes, but also from one hominid taxon to another.

We review here the evidence for the nature of incremental markings in tooth enamel, and consider the reasonable inferences which can be made from data on crown-formation times and the differing ways in which hominoid tooth crowns grow. We reassess the comparative developmental evidence available for early hominids, and highlight the main differences that exist between early hominid taxa. Finally, we have attempted to combine the available data on tooth-development times with the observed sequences of tooth development in the juvenile fossils to give a provisional chronology of dental development in early hominids.

Incremental growth markers in teeth

Teeth form incrementally with characteristic short- and long-period growth markings in enamel and dentine. In enamel, short-period markers (cross-striae or varicosities along enamel prisms) are generally accepted as representing daily increments, reflecting alterations in enamel secretion and composition during the circadian cycle^{8,14-18}. Longer-period markers, the striae of Retzius, represent successive surfaces of

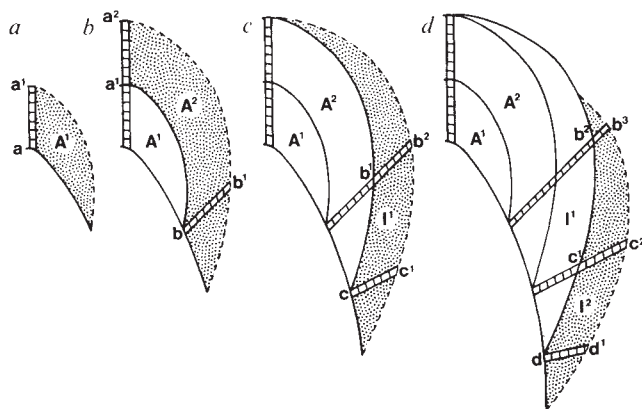


Fig. 1 Diagram showing the laying-down of successive enamel layers. Heavy interrupted line indicates forming enamel surface, which subsequently is represented by striae within the body of enamel. Attainment of full enamel thickness over the cusp tip involves successive layers of appositional enamel (A^1 , A^2), giving buried increments (that is, A^1) separated by striae (a^1 to b in second diagram). Subsequent layers on the side of the crown are imbricational layers (I^1 , I^2) which are associated with surface perikymata. The boundary between appositional and imbricational layers is indicated as $\cdots$. Enamel prism formation begins over the dentine horn (first diagram) from a to a^1 associated with the first appositional layer A^1 . In the second appositional layer A^2 (second diagram), the original prism is continued to the occlusal surface at a^2 , whilst concurrently a new prism [b to b^1] has begun cervically. This process is then repeated in imbricational enamel, with prism b extending from b^1 to b^2 in layer I^1 (third diagram), and so on through successive prisms c to c^2 , d to d^1 (fourth diagram).

the forming enamel (Fig. 1). In early formed enamel, layers are superimposed on earlier layers over the crown tip, such that the striae do not reach the surface. These layers represent hidden increments⁵. Subsequently, enamel layers are added on to the sides of the crown, building up the crown height in a tile-like fashion⁷. These striae do reach the crown surface, however, and give rise to ridges, or perikymata (Figs 1 and 2). There is some debate over the periodicity of striae, but there is considerable evidence, recently reviewed⁸, that suggests that in extant hominoids they reflect a circaseptan (7–9 day) rhythm.

The validity of a circaseptan cycle applicable in hominids

1–3 myr ago has been questioned¹¹. A practical problem is that striae are rarely observed in scanning electron micrographs of fractured fossil enamel, although the short-period (daily) varicosities are usually prominent. Nevertheless, stria can often be visualized using optical microscopy on fossil specimens immersed in ethanol¹⁹. The spacing between striae observed in this manner^{6,7,19} is $\sim 50\ \mu\text{m}$ in cuspal enamel and $30\ \mu\text{m}$ in cervical enamel, which is consistent with the observed range of varicosity spacing of ~ 6.0 and $3.5\ \mu\text{m}$, respectively, repeating over 7–9 days. This and other evidence^{7,8} supports the view that a circaseptan rhythm also operated during tooth formation in fossil hominids, thus allowing perikymata counts to be used to estimate the absolute time it has taken to form lateral imbricational enamel (Fig. 1). Good estimates can be made of the duration of hidden increment formation from observing naturally broken teeth or laboratory-prepared tooth sections.

Bromage and Dean⁵ used perikymata counts on the labial surfaces of incisor teeth to make estimates of crown-completion times in a small sample of fossil hominids: they concluded that incisor crown-formation times in *Australopithecus* were about twice as long as those of *Paranthropus*, and these data were then used to estimate ages at death. These were appreciably younger than previous estimates using modern human standards^{1,2}. Enamel-formation time is a function of two independent growth processes, one being associated with the daily incremental secretion rate by ameloblasts, and the other being dependent the enamel-extension rate²⁰, which reflects the differentiation of new cervical ameloblasts (Figs 1 and 2). In human deciduous teeth the extension rate is fast, allowing rapid completion of crown height. This involves the deposition of fewer imbricational layers, with correspondingly few perikymata (Fig. 2b). The pattern of rapid growth associated with a fast extension rate is also present in *Paranthropus* permanent incisors, premolars and molars^{6,19,21}. In permanent molar teeth in modern, and possibly early, *Homo* the extension rate is much slower, taking appreciably longer to complete the formation of crown height^{6,20}.

Estimates of molar crown-formation times in East African fossil hominids have been made by reconstructing patterns of crown growth using striae, and estimating crown-formation times using cross-striation data⁶. This study gave times shorter than would have been expected for large thick-enamelled posterior teeth as judged relative to molar formation times derived from previous radiographic studies on modern humans. The short formation time for the large thick-enamelled *Paranthropus* molars appears to be achieved by two separate mechanisms, a large daily incremental rate being accompanied by a fast extension rate (Fig. 2b). We have also reported⁷ a relatively

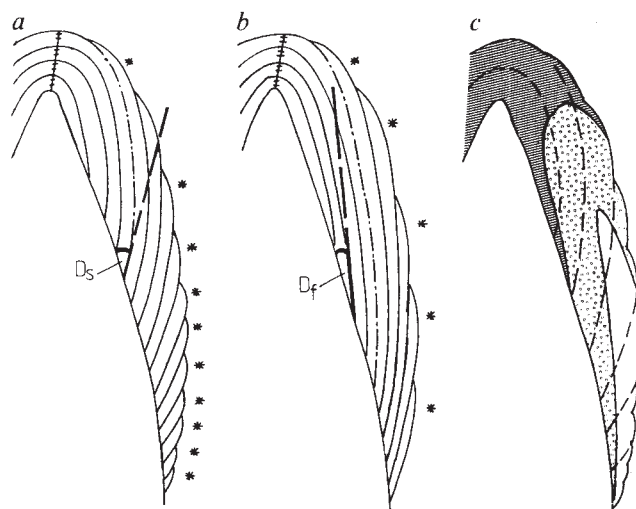


Fig. 2 Schematic diagram of enamel formation showing differences in crown formation times caused by a, slow and b, fast extension rates and c, the pattern of secondary mineralization (maturation) of enamel. The difference in slope of striae approaching the enamel-dentine junction (EDJ) reflect differences in enamel extension rates²⁰, with a larger angle (D_s) being associated with a slower extension rate and more enamel 'layers' and perikymata (*) for a given crown height (a), whereas the smaller angle (D_f) reflects faster extension rates, with fewer 'layers' and perikymata (*) for the same crown height, giving a shorter crown-completion time⁶. The junction between appositional and imbricational enamel is indicated — · — · — ·. Appositional enamel comprises a greater proportion of the crown area in teeth with a rapid extension rate⁶. The pattern of secondary mineralization of enamel (c) does not follow the incremental pattern shown in a (ref. 22). This process is initiated below the cusp tip beside the EDJ and spreads both outwards and cervically along the EDJ. Shading represents areas of relative mineralization, with the darkest shading being most highly mineralized.

brief crown-formation time of ~ 2.4 yr in a large thick-enamelled premolar tooth attributed to *Paranthropus*.

These histological studies have shown that hominid teeth, particularly in *Paranthropus*, grew in a manner different from those in modern man, and this difference should be considered when assessing radiographs of developing hominid teeth. For example, *Paranthropus* molar crowns half-way through their chronological growth period may have only 2 mm of cervical

Table 1 Perikymata counts and estimated mean crown-formation times for each tooth type

<i>Australopithecus</i>			<i>robustus</i>			<i>Paranthropus</i>			<i>boisei</i>			Early <i>Homo</i>		
Sts24a*	I ¹	135	SK62*	Right	I ₁ 57	OH30	I ₁	101	SK74b*	I ₁	110			
			SK62	Left	I ₁ 64	ER812	I ₁	86	ER820*	I ₂	105			
LH2*	I ₁	130	SK63		I ₁ 86	ER1477	I ₁	92	OH6	I ²	95			
LH3	I ¹	170	SK71		I ₁ >61	ER1820	I ₁	82	ER808	I ²	123			
LH3	I ²	180	SK73		I ₁ >79			$\bar{X} = 2.2$			$\bar{X} = 2.6$			
LH6	I ²	116			$\bar{X} = >1.9$									
	$\bar{X} = 3.3$													
LH3	C	168				OH30	C†	>103						
LH6	C	158				ER816A	C	109						
	$\bar{X} = 3.6$					ER1477	C†	>80						
								$\bar{X} = >2.4$						
LH3	P ³	95												
LH6	P ³	77												

I, incisor; C, canine; P, premolar; postscripts indicate tooth number and jaw. Crown-formation times (years) include 0.5 yr for appositional enamel formation time.

* Ref. 5.

† Crown incomplete.

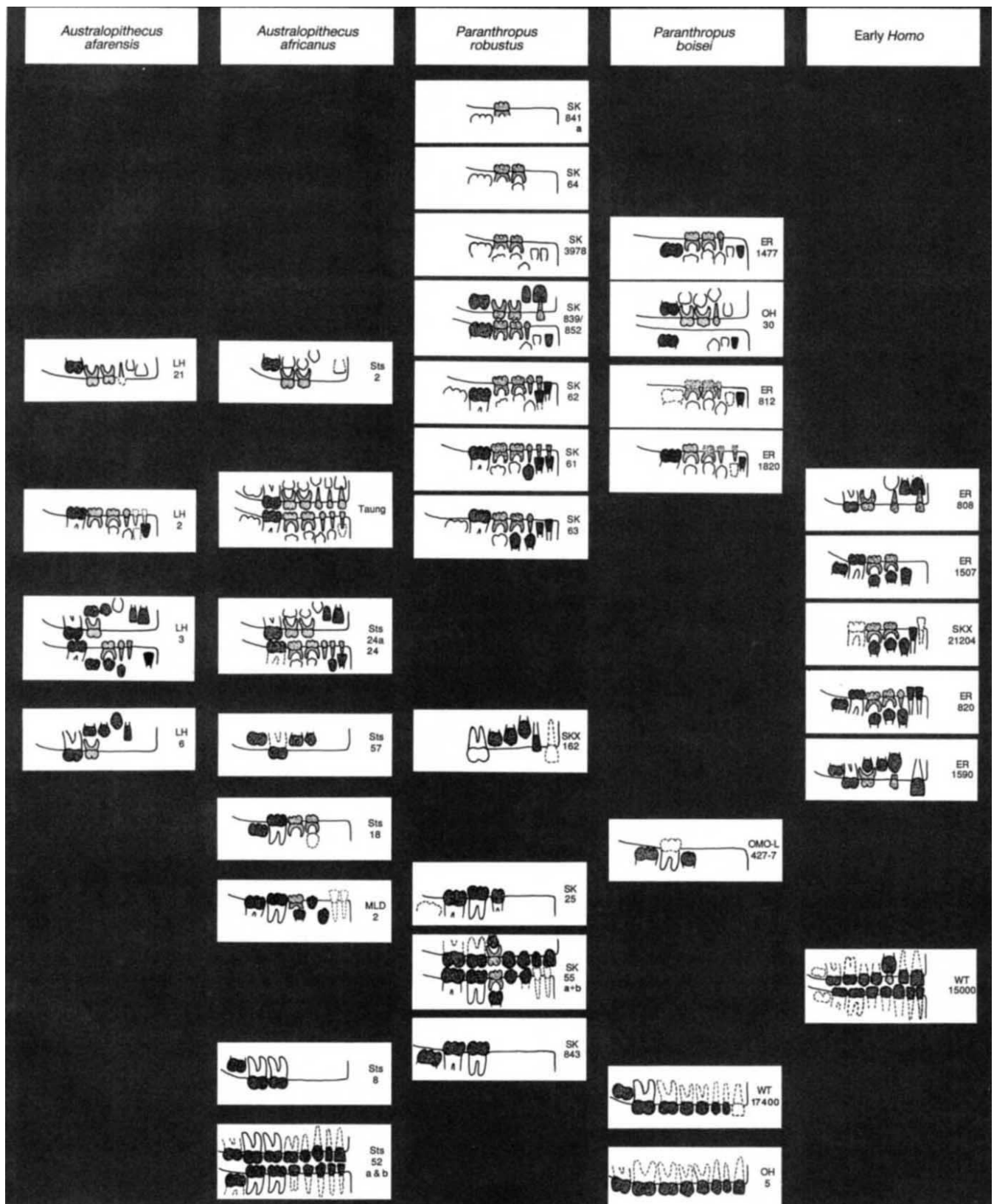


Fig. 3 Chart showing stages of dental development in juvenile fossil hominids documented in the literature (LH21⁴⁰; LH2, 3, 6⁴¹; Sts2⁴²; Taung^{29,43}; Sts24a & 24^{43,44}; Sts57⁴²; Sts18⁴²; MLD2⁴²; Sts8⁴²; Sts52a & b⁴²; SK841a³; SK64³; SK3978⁴²; SK839/852⁴⁵; SK62⁴²; SK61^{27,42}; SK63^{3,42}; SKX162⁴⁶; SK25⁴²; SK55a & b⁴²; SK843^{3,42}; ER1477²¹; OH30²¹; ER812²¹; ER1820²¹; OMO-L427-7⁴⁷; WT17400⁴⁸; OH5⁴⁹; ER808^{43,50}; ER1507²¹; SKX21204⁵¹; ER820²¹; ER1590⁵⁰; WT15000⁵⁰). Only specimens with evidence of first permanent molar (M_1) development plus one or more other teeth in jaws or in related tooth collections are included. Specimens are calibrated in developmental series within and between the columns on the basis of their M_1 development. Specimens with the same M_1 development and emergence are ranked relative to the developmental status of the other teeth. Incomplete teeth are indicated by incomplete outlines; completed permanent crowns are shaded, deciduous teeth are stippled. Stages of incomplete crown and root formation are proportional representations. Teeth which are absent or for which no data are available, but whose stages of development can reasonably be inferred, are indicated in interrupted outline. Teeth are represented without distinction of side.

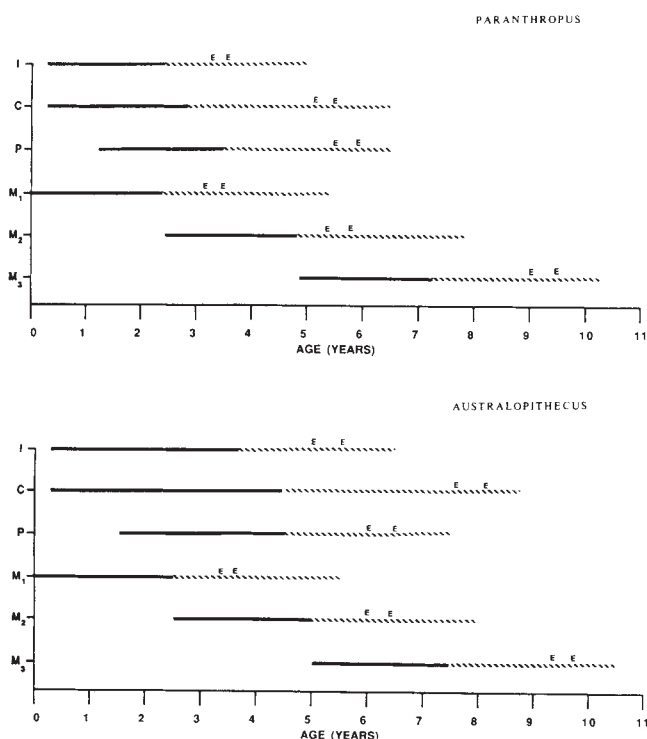


Fig. 4 Chronology of dental development in *Australopithecus* and *Paranthropus*. Period of crown formation is shown by a heavy unbroken line and period of root formation by a broken line. E-E, Estimated range of tooth emergence. Tooth type (see Table 1) is indicated on the left. Molar crown formation times in *Australopithecus* are estimated to be similar to those in *Paranthropus* at 2.5 yr. In both taxa it is assumed that M_1 crown calcification begins at birth; that M_2 crown calcification begins as M_1 crown formation is completed; and that M_3 crown calcification commences as M_2 crown formation completes. Root formation times are judged from coincident stages of crown and root formation on X-rays, computerized tomography scans, or as observed on original specimens (see Fig. 3), and are assumed to be similar in each tooth type.

enamel remaining to form, but may easily be misinterpreted in radiographs as being nearly complete, as the occlusal enamel is already thick, giving the appearance of maturity.

A further complication arises when assessing stages of crown development on radiographs, as a result of the pattern of secondary mineralization, or maturation of enamel. This process does not follow the incremental deposition of enamel, but extends along the enamel-dentine junction early in amelogenesis, maturing outward towards the surface²² (Fig. 2c). Almost all radiographic studies (see ref. 9, 12 and 13) assume that crown formation occurs in a regular linear manner, but there is accumulating evidence that the rate of enamel formation slows progressively towards the cervical region^{23,24}. These two aspects of enamel growth, of cervical slowing and the unusual pattern of maturation, make it difficult to determine the precise stage of crown formation, particularly near completion, in radiographs of developing jaws. Such estimates are also unreliable since the last formed thin enamel on the buccal aspect is 'burned out' on radiographs, allowing only earlier completing mesial and distal enamel to be used to judge crown completion.

It is also clear from studies of perikymata counts that there are marked differences in extension rates in anterior teeth between *Paranthropus* and *Australopithecus*. *Paranthropus* shows a crown extension rate which is nearly linear, being manifested by relatively evenly spaced perikymata, whereas *Australopithecus* shows progressive slowing of extension rates, with reduced spacing between cervical striae²¹. For example, the most cervical 2 mm of enamel in two *Australopithecus* speci-

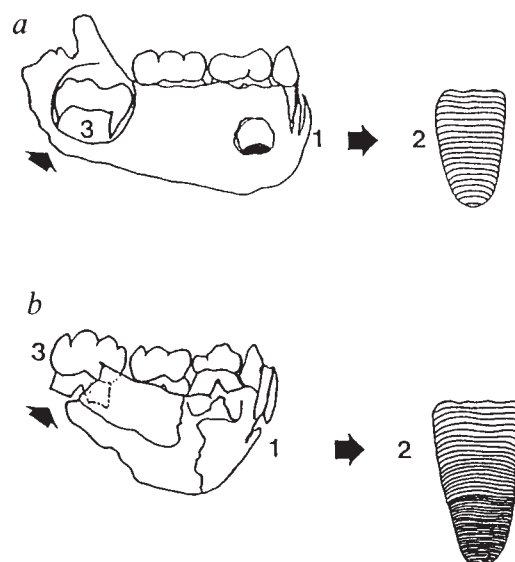


Fig. 5 Incisor/molar developmental relationships. *a*, *Paranthropus* (KNM-ER 1477). The central incisor (1) has a completed crown with 92 perikymata (2), indicating a period of ~1.7 yr for imbricational enamel formation, plus 0.5 yr for appositional enamel formation, plus an estimated 0.5 yr delay in calcification after birth, indicating an age of about 2.7 yr. The M_1 crown (3) is complete as viewed in a radiograph, and another estimate of molar crown formation gives an age of ~2.5 yr⁶. These values are concordant, and indicate early completion of I_1 and M_1 crowns. SK 63 is at a slightly later stage, with crown completion and some root development of the I_1 , I_2 , C and P_3 , and shows eruption and functional occlusion of M_1 , suggesting early eruption of the M_1 . The M_2 crown is half-complete. *b*, *Australopithecus* (LH 2). The unerupted central incisor (1) shows 130 perikymata (2), indicating a period ~2.5 yr for imbricational enamel, plus 1.0 yr for appositional enamel and delay (see above), indicating an absolute age at death of ~3.5 yr. The M_1 has completed crown formation, and has erupted into occlusion having formed its root bifurcation (3). Taung is at a similar stage, with development of half of the M_2 crown, which will complete in <1.5 yr.

mens (LH2 and Sts24) contains 60 perikymata, taking more than a year to form. In contrast, the whole of the imbricational enamel in *Paranthropus* is completed in the same time.

Sequences of dental development in hominids

There has been controversy about differences in the sequence of dental development in hominids since the discovery of Taung in 1925. Over the years there have been repeated attempts to compare dental development and eruption sequences in hominids with those documented in modern man or great apes^{2,3,9,25-27}. There have been numerous radiographic studies on dental development in modern man, but there is still controversy over which of these studies present the most appropriate comparative material^{12,13}. In contrast, there are relatively limited data on dental development in great apes²⁸.

The stages of dental development in the dentition of the Taung 'child' have recently been described using computerized tomography²⁹. These authors concluded that this important specimen shows developmental affinities with modern apes, although this conclusion has been disputed³⁰. Because of this controversy about human developmental standards and the paucity of ape data, we consider it is premature to make detailed comparisons of dental development between early hominids, humans and great apes. It may also be misleading to make such direct comparisons without due regard to the interrelationships between jaw and tooth growth in hominoids. There are specific differences in dental development and eruption between modern apes and man³¹, which are almost certainly a consequence of

space relationships within the developing jaws. Modern apes have both large canines and diastemata, and the canines develop slowly and erupt very late, near the end of the relatively shorter growth period. This delay probably reflects the necessity for sufficient time to permit jaw growth to occur to accommodate both a large canine tooth and a diastema in each jaw quadrant. In modern man the canines are relatively small and there are no diastemata; the canines develop earlier, erupting roughly two-thirds through the extended human growth period. Small canines and the absence of diastemata also characterize later hominids. Relatively little is known about facial growth in hominids, but the available evidence suggests there are major differences between early hominid taxa³². The persistent efforts to compare hominids with modern man or apes also distract attention from differences that are present between the two main hominid genera *Australopithecus* and *Paranthropus*.

The stages of dental development in juvenile hominid jaws are difficult to determine precisely, and the descriptions are scattered widely through the literature. We present the data in chart form from juvenile hominid specimens which have the developing first molar (M_1), together with detailed developmental stages in other teeth (Fig. 3). Specimens are grouped into columns representing five hominid taxa and are calibrated relative to the development of the M_1 . The youngest specimens are represented at the top of each column, with successively older specimens ordered in sequence below. Evidence of tooth function, shown by wear facets, was used in the ranking of specimens. Several specimens have not been included because of lack of appropriate data on tooth development. Specimens of different taxa are located at comparable horizontal levels relative to their M_1 developmental stage. There is a consistent difference between species belonging to *Australopithecus* on the one hand, and to *Paranthropus* on the other. In *Paranthropus*, there is parallel development of M_1 and the incisor I_1 , with both teeth completing crown formation together. In contrast, in *Australopithecus* incisor crowns are not yet complete, even when the M_1 is in functional occlusion. Although there are only six juvenile specimens attributed to early *Homo*, it appears that molars and incisors are also emerging close together in the growth period. This comparison of data from *Australopithecus* and *Paranthropus* supports the view that there are consistent major differences in dental development between *Australopithecus* and *Paranthropus*.

Chronology of dental development in hominids

We present an extended list of perikymata counts and estimated crown formation times in Table 1. We have used these data together with data from other incremental studies^{6,7} to construct a list of crown-formation times in three fossil hominid taxa (Table 2).

In all living hominoids the M_1 calcifies at about the time of birth, with incisors calcifying soon afterwards during the first year of life^{33,35}, and it is probable that this was also the case in fossil hominids. This assumption, together with the observed sequences of tooth development (Fig. 3), was used to construct a provisional chronological chart of dental development in *Australopithecus* and *Paranthropus* (Fig. 4). Examples of the approach used to construct this chart are illustrated in Fig. 5. The data presently available on early *Homo* are too scant to construct a similar chronology. There is a consistency between estimates of crown-formation times in anterior teeth using perikymata counts, and those in posterior teeth using different internal growth indicators. Incisor and molar completion times in *Paranthropus* are concordant^{5,6,21}, and fit the observed dental developmental and eruption sequences (Fig. 5a). In *Australopithecus* the incisor crowns take an appreciably longer period to form. During this same period, the first molar has not only completed its crown formation, but has erupted into occlusion, having formed its root bifurcation (Fig. 5b). This latter observation suggests that root growth in fossil hominids

Table 2 Crown-formation times in years in different hominid taxa

	<i>Australopithecus</i>	<i>Paranthropus boisei</i>	Early <i>homo</i>
Incisor	3.3	2.2	2.6
Canine	3.6	2.6	?
Premolar	3.0*	2.4*	?
Molar	?	2.4†	2.5†

Ages for incisor and canine teeth are from perikymata counts using a 7-day periodicity and allowing 0.5 yr for 'hidden' increments.

* *Australopithecus* premolar age is obtained from perikymata counts as above, summed with time to form appositional enamel (1.3 yr) in human premolars, using unpublished data²⁴ and data for *Paranthropus* premolar⁷.

† Data for *Paranthropus* and *Homo* molar from ref. 6.

was rapid, being associated with early tooth eruption, and is supported by morphological evidence on developing roots^{26,28}.

The early emergence of the M_1 into occlusion in fossil hominids is significant, as it has been shown in all living primates that the eruption of the M_1 into occlusion coincides with the attainment of 95% of adult cranial capacity^{34,35}. This is consistent with the absolutely small brain size of early hominids³⁶. The estimate of M_1 eruption at 3.5 yr in *Australopithecus*, and possibly even earlier in *Paranthropus* is within the modern great ape range, but is about 5 standard deviations below modern human mean values³⁷. Although both hominid taxa appear to complete their dental development within a similar period of time there are distinct differences in dental development between the two taxa. These include the combination of developmental sequences, crown extension rates and anterior tooth crown formation times. We believe these differences are beyond those typically observed within a single hominoid genus, and so support the original proposal for separate generic status by Broom³⁸ and Robinson³⁹. We are aware, however, that this conclusion is not universally accepted.

Conclusions

This preliminary chronological reconstruction of dental growth and development in *Australopithecus* and *Paranthropus* suggests that the overall period of growth and development was short. Although both hominid taxa appear to complete their dental development within a similar period of time, there are distinct differences in the sequences of dental development between the two taxa. The shorter period of dental development in fossil hominids indicated by the tooth-formation times obtained from incremental markings combined with developmental sequences, suggests that the significant stage in human evolution that is characterized by a prolongation of the childhood growth period had not yet begun.

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ARTICLES

Chemistry of hot springs on the Mid-Atlantic Ridge

A. C. Campbell*, M. R. Palmer*, G. P. Klinkhammer*, T. S. Bowers*, J. M. Edmond*, J. R. Lawrence†, J. F. Casey‡, G. Thompson‡, S. Humphris‡, P. Rona§ & J. A. Karson||

* Department of Earth, Atmospheric and Planetary Sciences, Massachusetts Institute of Technology, E34-201, Cambridge, Massachusetts 02139, USA

† Geologic Sciences, University of Houston, Houston, Texas 77004, USA

‡ Department of Chemistry, WHOI, Woods Hole, Massachusetts 02543, USA

§ NOAA/AMOL, 4301 Rickenbacker Causeway, Miami, Florida 33149, USA

|| Department of Geology, Duke University, Durham, North Carolina 27706, USA

The first hydrothermal fluid samples collected along the slow-spreading Mid-Atlantic Ridge (MAR) are remarkably similar in composition and temperature to fluids collected along the shallower, faster-spreading East Pacific Rise (EPR). The MAR fluids, like those from the EPR, appear to be in equilibrium with a greenschist-facies mineral assemblage. In contrast to the EPR, the more fractured nature of the MAR apparently allows fluids at one of the MAR sites to interact with weathered basalt.

SINCE the original discoveries of hydrothermal activity on oceanic spreading centres in the Eastern Pacific in 1977 and 1979^{1,2}, hot springs have been found at numerous locations on the East Pacific Rise (EPR)^{3–5} and on the Juan de Fuca and Explorer Ridges^{6,7}. These ridge systems all have intermediate to fast spreading rates (6–12 cm yr⁻¹) and fluid samples from them display a wide range in temperature and chemical composition^{8–13}. Until recently there was no direct evidence for high-temperature venting activity on slow-spreading centres such as the Mid-Atlantic Ridge (MAR). During the summer of 1985 hot springs were discovered at two sites in this region, the Trans-Atlantic Geotraverse (TAG) area at 26°08'N (ref. 14) and the Mid-Atlantic Ridge at Kane (MARK) site at 23°22'N (ref. 15). Both sites are at depths of ~3,500–3,700 m, over a kilometre deeper than those on the EPR. The magmatic heat source driving the hydrothermal activity on slow-spreading ridges is thought to be 2–3 km deeper in the crust than at fast-spreading ridges¹⁶. This led to the hypothesis that hydrothermal fluids circulating in the reaction zones on the MAR would originate from a regime of higher temperature and pressure than on the EPR. The chemistry of the hydrothermal fluids would reflect these differences by exhibiting much more dissolved silica (because of increased solubility of quartz) and greater metal concentrations.

Contrary to this hypothesis, samples collected during four reconnaissance ALVIN dives on both sites in the spring of 1986 have compositions and exit temperatures surprisingly similar to those observed on the EPR. Calculated depths of reaction based on quartz geobarometry are less than 1–2 km in the crust (~450–

550 bar hydrostatic pressure). Thermodynamic equilibrium calculations indicate that the factors controlling the major element chemistry of hydrothermal fluids are similar for both the EPR and MAR. Alkali element ratios, boron isotope and rare earth element (REE) data indicate that MAR fluids have reacted with both weathered and fresh basalt. The discovery that the exit temperatures are no higher than 350 °C, despite the greater hydrostatic pressure, suggests that the practical upper limit to fluid temperatures for seafloor hot springs is determined by the transport properties of water and the rates at which secondary mineral precipitation heals fractures. These processes may restrict fluid flow through the higher-temperature regions of the reaction zone.

Geological setting

The TAG site lies in a typical rift valley setting on the slow-spreading MAR about 275 km north of the Kane fracture zone. Numerous expeditions to the area have recovered metalliferous sediments and pure MnO₂ crusts from the eastern wall of the rift¹⁷. Both temperature and ³He anomalies have been observed in the water column¹⁸. The active high-temperature field is found on the floor of the rift near the base of the east wall. This site is ~1.5 km from the neovolcanic zone that marks the current spreading axis and is on crust estimated to be 100,000 years old (half-spreading rate = 1.3 cm yr⁻¹ (ref. 14)). It was located by following the gradients in the water-column anomalies of Mn, temperature and particulate material^{19,20}. The field is visible on a Seabeam map of the area as an elliptical mound about 50 m

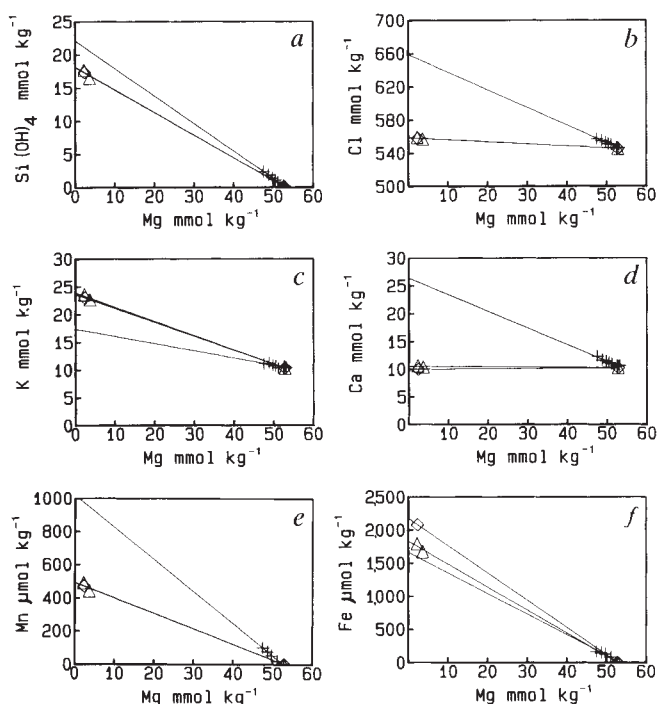


Fig. 1 Fluid concentrations of *a*, Si(OH)_4 , *b*, Cl, *c*, K, *d*, Ca, *e*, Mn and *f*, Fe against Mg for the TAG and MARK hydrothermal vents. Linear regression lines used to calculate the end-member concentrations are also shown. Concentration units are per kg of solution. Plot symbols: + TAG; ◇ MARK-1; △ MARK-2.

high and 200–250 m in diameter. Three reconnaissance dives showed this mound to be entirely covered with hydrothermal sulphide precipitates, their oxidation products and anhydrite²¹.

Seeps of warm- and intermediate-temperature water are ubiquitous in the area. High-temperature venting was observed at three locations on the mound. A field of onion-shaped, sphalerite-rich chimneys, emitting copious amounts of light blue 'smoke', was encountered near the outer edge of the mound. Near the base of the mound's central peak are numerous black smoke seeps with water temperatures of 290–321 °C. This second area yielded fluids diluted with about 90% ambient water as a result of the lack of well defined orifices and the diffuse nature of the flow. In the centre of the mound there is a tall (>10 m) edifice 30–40 m in diameter, which is constructed from a large number of coalesced active chimneys that are mainly composed of pyrite, marcasite, chalcopyrite and anhydrite²¹. Diffuse flows of black smoke bellow from the flanks of this structure, making access to the well developed orifices on the top extremely difficult. Diluted samples were recovered from the flank seeps. The mass of hydrothermal precipitates present could be several million tons, if the entire mound is primarily composed of sulphides and lesser amounts of anhydrite¹⁴. This would make the TAG area the first active deposit found on the sea floor that is comparable in size to the ore bodies exploited in ophiolites.

The MARK vent field is 25 km south of the Kane Fracture Zone, in an area identified as a potential hydrothermal site during a SEAMARC survey²² and subsequently located by the Ocean Drilling Program's drill ship *Joides Resolution*¹⁵. It was visited on a single ALVIN dive and is on glassy pillows in the middle of the neovolcanic zone atop a large volcanic ridge 600 m high, 4 km wide and 40 km long²³. The area of high-temperature activity is a linear ridge of active coalesced chimneys and sulphide debris ~30 m high and 75–100 m long. Two other parallel ridges were found to the west of this active chain. The central ridge was inactive, while the westernmost one had low-temperature seeps and white smoker vents (226 °C). The slopes of the ridges are covered with talus composed of sulphide blocks

and mantled with bright orange, oxidized metalliferous sediments. The troughs between the ridges are also covered with metalliferous sediment. The 5–10-m-high chimneys on top of the east ridge have well developed orifices that discharge water at temperatures of 350 °C; an excellent fluid sample was obtained from one of them. Approximately 10 m deeper, on the flank of the ridge, an active onion-shaped chimney was collected which continued to emit black smoke after being toppled into ALVIN's sample basket. The feeder vent to this structure gave a stable temperature of 335 °C and also provided good fluid samples.

Fluid chemistry

The procedures used for data collection and analysis were similar to those described previously^{9,12}. The fluids, collected in titanium syringe samplers, were drawn onboard the ship shortly after recovery of ALVIN. Measurements of pH, alkalinity and H_2S were completed within 2–3 h and Si(OH)_4 , Fe and Mn were analysed within 12 h of recovery. These data were used as a guide to the success of the sampling procedures and to provide any indications of unusual properties relative to EPR fluids. In addition to the analytical methods used in previous work, isotope-dilution thermal-ionization mass spectrometry was used to analyse K, Rb, Cs, Sr and $^{87}/^{86}\text{Sr}$. Boron and boron isotopes were determined as in ref. 24. The REE were measured by isotope-dilution mass spectrometry as part of a joint project with H. Elderfield and M. Greaves at the University of Cambridge. The calculated end-member compositions of all the data are given in Table 1 and the nature of the extrapolations used to obtain them are illustrated for Si(OH)_4 , Cl, K, Ca, Mn and Fe in Fig. 1.

The TAG samples are ≤10% hydrothermal fluid and only the major-element data are presented in Table 1. The Cl, Na, Ca and Sr values are higher than at 21° N EPR^{9,12}, but within the range of the fluids collected between 13° N and 11° N EPR^{10,13}. The Li, K and Rb values for the TAG fluids are significantly lower than those for most other hydrothermal systems. The Cs/Rb mole ratios of the TAG hydrothermal fluids (10×10^{-3}), like those from 21° N and 11–13° N EPR ($8\text{--}11 \times 10^{-3}$), are similar to Cs/Rb ratios measured in fresh mid-ocean-ridge basalts (MORB) (8×10^{-3}). Both the iron and manganese concentrations are similar to EPR fluids.

The MARK samples are equivalent in quality (that is, lack of dilution with ambient waters) to some of the best of the EPR samples^{9,12}. Two individual vents were sampled within the MARK area and they are reported separately in Table 1. The major-element data are similar to the fluids from the Ocean Bottom Seismometer (OBS) vent at 21° N EPR (see Table 1). The end-member concentrations of Li, K and Cs are higher than at TAG, but Rb is approximately the same. The lower Ca and Sr concentrations at MARK relative to TAG reflect the lower chloride content of the MARK fluids. These alkaline earth elements have been shown to correlate positively with chloride for the EPR and Juan de Fuca systems¹³. Chloro-complexation shifts the equilibrium between a secondary mineral phase, containing Ca and Sr (epidote), and the fluid phase toward greater concentrations in solution. The $^{87}/^{86}\text{Sr}$ values for both TAG and MARK are the same as for fresh MORB. Iron and manganese are both within the range of values from 21° N (refs 9, 12). With the exception of iron and copper, the major-element data and the isotope data do not show any significant differences between MARK-1 and MARK-2. The ~350 $\mu\text{mol kg}^{-1}$ difference in iron and 7 $\mu\text{mol kg}^{-1}$ difference in copper between the two vents at MARK are larger than their respective combined analytical and regression errors. The silica concentrations are about 1 mmol kg^{-1} higher than for OBS, South West (SW) and Hanging Gardens (HG) vent fields at 21° N EPR. Since the temperature of these systems are all ~350 °C, the higher values for MARK are probably due to the greater depth of this system. The estimated depth of the reaction zone is ~1–2 km below the sea floor at the MARK site (determined by quartz geobarometry

Table 1 End-member fluid values for the TAG and MARK vents on the Mid-Atlantic Ridge compared to the OBS vent at 21° N EPR

	TAG	MARK-1	MARK-2	OBS 21° N (refs 9, 12)
Temperature (°C)	321–290	350	335	350
pH*		3.9	3.7	3.4
Alkalinity ($\mu\text{mol dm}^{-3}$)		–64	–243	–400
Si(OH) ₄ (mmol kg^{-1}) [†]	22.0	18.2	18.3	17.5
H ₂ S (mmol kg^{-1})		5.9	5.9	7.3
Cl (mmol kg^{-1})	659	559	559	489
Na (mmol kg^{-1}) [‡]	584	510	509	432
Li ($\mu\text{mol kg}^{-1}$)	411	843	849	926
K (mmol kg^{-1})	17.0	23.6	23.9	23.5
Rb ($\mu\text{mol kg}^{-1}$)	10	10.5	10.8	23.5
Cs (nmol kg^{-1})	100	177	181	202
Be (nmol kg^{-1})		38.5	38.0	15
Mg (mmol kg^{-1})	0	0	0	0
Ca (mmol kg^{-1})	26.0	9.9	10.5	15.6
Sr ($\mu\text{mol kg}^{-1}$)	99	50	51	80
Sr (87/86) [§]	0.7029	0.7028	0.7028	0.7034
Mn ($\mu\text{mol kg}^{-1}$)	1,000	491	493	1,024
Fe ($\mu\text{mol kg}^{-1}$)	1,640	2,180	1,832	1,530
Cu ($\mu\text{mol kg}^{-1}$)		17	10	35
Zn ($\mu\text{mol kg}^{-1}$)		50	47	106
Al ($\mu\text{mol kg}^{-1}$)		5.3	5.0	5.2
B ($\mu\text{mol kg}^{-1}$)		518	530	500
$\delta^{11}\text{B}$ (‰)		+26.8	+26.5	+32.2 (ref. 24)
$\delta^{18}\text{O}$ (‰) [¶]		+2.37	+2.37	+1.6 (ref. 38)
$\delta^{34}\text{S}$ (‰) [#]		+4.9	+5.0	+1.5 (ref. 41)
La (nmol kg^{-1})		2,700	1,670	900
Sm (nmol kg^{-1})		430	428	96
Eu ^{*††}		1.19	1.16	1.50
La _N /Sm _N ^{‡‡}		3.6	2.2	5.8

* NBS buffer scale. † Concentration units are per kilogram of solution. ‡ Charge balance sodium. § Normalized to E&A Std = 0.7080. || Relative to NBS SRM 951. ¶ Relative to SMOW. # Relative to CDT (W. C. Shanks III, personal communication). †† Eu^{*} defined in the text. ‡‡ Normalized to chondrite.

using the quartz solubility curves of von Damm and Bischoff¹¹). The ionic strength of the MARK fluids (0.573) is 17% lower than the TAG fluids (0.688). The differences in major-element chemistry between the TAG and MARK regions are no greater than the variability found for vent fields along the EPR^{9,10,12,13}.

Fluid temperatures

All the temperature measurements on the expedition are internally consistent as they were done with the same thermocouple probe and electronics package. Replicate temperature measurements were made by allowing the probe to cool to ambient values between each reading and were reproducible to $\pm 2^\circ\text{C}$ or better. The temperature readings at TAG must be considered minimum values as they were taken in diffuse flows. The 15 °C difference between MARK-1 and MARK-2 is significant, because readings at each vent were done in duplicate with the same probe on the same dive. A recent calibration check of two ALVIN high-temperature probes with a high-precision gas-chromatograph oven and independent thermometers and thermocouples shows that the ALVIN probes have an accuracy of better than $\pm 1\%$ up to 400 °C (Campbell *et al.*, in preparation).

Campbell *et al.*¹² noted that, in general, the exit temperatures and chemical compositions of seafloor hot springs are remarkably stable on a timescale of several years. Between 1981 and 1985 at the 21° N EPR hydrothermal site only one of the four vent fields (HG) showed any discernable changes. This vent appeared to have cooled $\sim 10^\circ\text{C}$ relative to the others and the reaction zone became ~ 1 km deeper in the crust. Dissolved iron decreased by 50% and manganese by $\sim 30\%$. In contrast, major elements like Li, K, Ca and Sr were unchanged over time at the same vent site. These authors suggested that iron is a sensitive indicator of changes in vent fluid chemistry and temperature. It is important to note that this trend is only true for fluids from the same aquifer; fluids from different aquifers display a wide range in major-element and minor-element composition

although they may be all at the same temperature^{9–13}. Copper concentrations in vent fluids are also sensitive to temperature, whereas zinc is apparently controlled by other factors^{9,11}.

The fact that iron and copper concentrations are lower in the cooler solutions from MARK-2 while the other elements show no significant differences from MARK-1, suggests that fluids from these two vents are derived from the same aquifer. But the waters debouching at MARK-2 have cooled in the near-surface

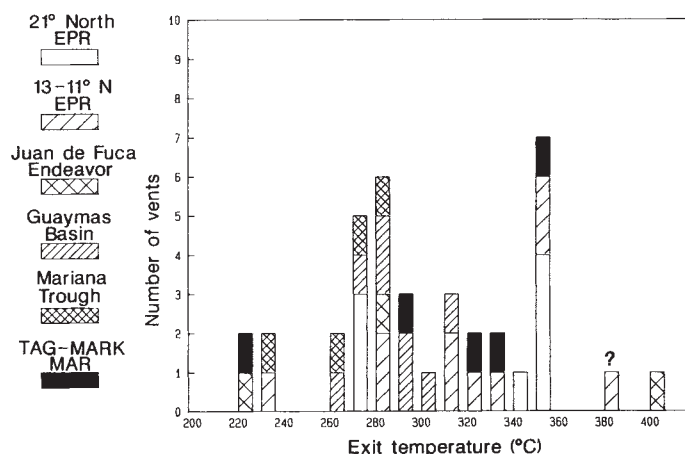


Fig. 2 Histogram of exit temperatures measured at individual hydrothermal vents. Replicate measurements have been made at a number of the vents. The 21° N and Guaymas Basin data are reproducible over several years of a time series¹². Only one value is plotted for any one vent. The value for 13° N shown with a ? is a single measurement and reviews of the dive tape suggested that it was erroneously high because of case heating^{12,13}. References for these data are as follows: 21° N EPR^{9,12}, 11–13° N EPR^{4,10,13}, Juan de Fuca¹¹, Endeavor²⁵, Guaymas Basin⁴², Mariana Trough⁴³ and TAG-MARK (this work).

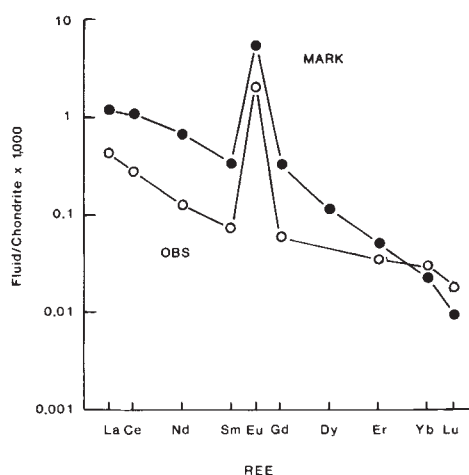


Fig. 3 REE end-member fluid concentrations normalized to chondrite against atomic number for the MARK-1 and OBS 21° N EPR vent sites.

region, perhaps in the mound plumbing system. This cooling is not due to sea water mixing with the vent fluid in the sub-surface region: if ~4% sea water were to mix with the fluid to produce the 15 °C decrease, then the $^{87}/^{86}\text{Sr}$ ratio for MARK-2 would be 0.00025% heavier than for MARK-1. This change is larger than the standard error of the Sr isotope measurements and the two vents have virtually identical $^{87}/^{86}\text{Sr}$ values. The black smokers are on top of a 30-m thick ridge of sulphide rubble^{15,21} and secondary circulation cells within and beneath this structure may provide a heat sink sufficient to cool the flank vent (MARK-2) relative to the summit vent (MARK-1).

There is considerable debate in the literature about the role that phase separation may play in determining the upper temperature limit and composition of seafloor vent fluids^{11,13,25-28}. A compilation of measured exit temperatures for seafloor hydrothermal fluids is presented in Fig. 2. Virtually all of the reported values are less than or equal to 350 ± 5 °C. It is unlikely that the steep fall-off above 350 °C is a statistical aberration. It is more likely, given the preference for sampling the hottest fluids, that the lower-temperature fluids are under-represented. Because the MARK vents are ~1 km deeper than the EPR and Juan de Fuca systems the two-phase boundary for these fluids is ~40 °C higher than for the shallower systems^{27,28}. The deeper systems could attain much higher exit temperatures than the shallower systems if phase separation determines the upper limit of hydrothermal fluid temperatures. The fact that virtually all the measured exit temperatures are no higher than ~350 °C for these systems (well below the two-phase boundary for their respective pressure regimes²⁷) suggests that this may be a practical upper limit to fluid temperature in deep systems.

The rate at which minerals precipitate at high temperature and pressure may limit the porosity of the reaction zone. Quartz solubility in seawater solutions reaches a maximum at temperatures below the two-phase boundary at the hydrostatic pressures found in most sub-seafloor hydrothermal systems¹¹. Recent experimental work on the healing rate of fractures in quartz, at elevated temperatures and pressures shows that this process is a positive function of temperature, pressure and ionic strength²⁹. The healing rates range from tens of hours to tens of weeks at the temperatures and pressures thought to occur in sub-seafloor hydrothermal systems. Other secondary mineral phases may behave in a similar fashion. The transport properties of water also reach a maximum near 350 °C (ref. 30). Thus the prevalence of exit temperatures below 360 °C may be a function of the physico-chemical properties of water, and mineral precipitation would limit the water volume that can flow through the higher-temperature regions of the reaction zone.

Table 2 Calculated pH and affinities for MARK vents compared to EPR

	MARK-1	MARK-2	21°N and 11-13°N EPR ¹³
Temperature*	350 °C	350 °C	350 °C
pH	4.61	4.38	4.1 to 4.7
Affinities (kcal)			
Quartz	0.08	0.09	0.0 to 0.6
K-feldspar	-4.90	-6.38	-6.2 to -2.1
Albite	-3.61	-5.11	-4.8 to -0.5
Anorthite	-8.23	-11.17	-8.3 to -1.0
Muscovite	-6.96	-10.15	-6.9 to 0.6
Paragonite	-7.33	-10.54	-7.1 to 0.6
Ca-beidellite	-4.39	-6.56	-3.1 to 2.4
Epidote	-11.23	-16.98	-16.4 to -3.5
Daphnite	2.18	-7.25	-12.5 to 2.9
Pyrrhotite	-0.20	-1.74	-3.4 to -1.1

*Temperature used in calculations.

Thermodynamic calculations

A thermodynamic analysis of the MARK vent fluids was done in a manner similar to the approach used for modelling the EPR vents¹³. Table 2 shows the calculated *in situ* pH values and affinities (in kcal) for several minerals for the MARK-1 and MARK-2 fluids at 350 °C and 500 bar. We chose this pressure rather than the 250 bar used for the EPR calculations because the MARK site is ~1 km deeper than the EPR sites. Databases for pressures between 250 and 500 bar are not currently available. Methods and thermodynamic data used here are the same as in Bowers *et al.*¹³, where they are discussed in detail. Although the pH values at 25 °C for the MARK vents are higher than for the EPR vents, the calculated high-temperature pH values fall within the range calculated for the EPR vents. Likewise, the calculated affinities for several greenschist assemblage minerals at MARK site fall within or close to the range calculated for the EPR vents at 350 °C. These results imply that, like the vents along the EPR at 21° N and 13-11° N, the major-element chemistry of the MARK fluids is controlled by equilibrium thermodynamics and saturation with respect to a suite of greenschist assemblage minerals in the altered basalt.

Fluid-rock interactions

Previous studies of hydrothermal fluids from the EPR and Juan de Fuca have assumed that the high-temperature solutions have only reacted with fresh basalt^{9,11,24,31}. B, Cs and Rb are thought to be quantitatively extracted from basalt during hydrothermal reactions without subsequent re-equilibration with secondary mineral phases^{9,24}. The relative concentrations of these elements in the fluids (after correction for water loss from hydration of the crust and original seawater concentrations) should reflect those in the basalt from which they were leached. The relatively low Rb concentrations at MARK and TAG appear to reflect the depleted nature of basalts in this section of the Mid-Atlantic Ridge³². Mole ratios for Cs/Rb are relatively constant in MORB ($\sim 8 \times 10^{-3}$) (ref. 33) and most vent fluids have similar values ($8-11 \times 10^{-3}$). In contrast, the Cs/Rb ratio of the MARK fluids (17×10^{-3}) is distinctly higher than MORB values and instead lies in the range described by weathered basalts ($\sim 25 \times 10^{-3}$), suggesting that the MARK fluids have reacted with weathered basalts.

Boron is very sensitive to reactions with altered basalts because it is present in sea water in relatively high concentrations and is rapidly taken up during low-temperature (<150 °C) weathering of oceanic crust²⁴. Data from the EPR were modelled in terms of little or no removal of boron from sea water on the cold down-welling limb of the hydrothermal convection cell

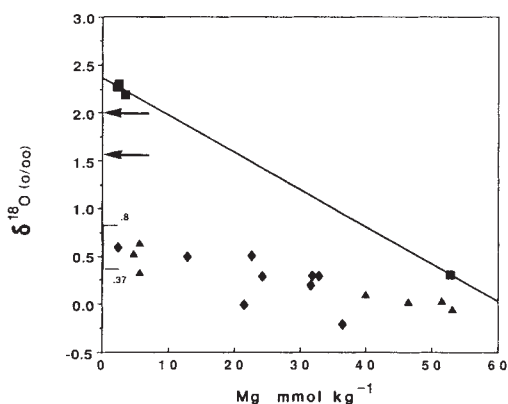


Fig. 4 Oxygen isotope compositions of MARK hydrothermal fluids compared to Juan de Fuca, 13° N EPR and 21° N EPR data. The extrapolated end-member for MARK is 2.37‰. The end-member values for 21° N EPR (shown by the arrows) range from 1.6‰ to 2.0‰. The end-member values for Juan de Fuca and 13° N EPR range from 0.37‰ to 0.8‰. The symbols and data references are as follows: ■ MARK (this work); ← 21° N EPR^{38,31}; ♦ Juan de Fuca³⁹; ▲ 13° N EPR⁴⁰.

followed by quantitative extraction of boron from fresh MORB during high-temperature water-rock reactions²⁴. Application of the same model to the MARK data suggests that at least 40% of the boron is removed during low-temperature weathering reactions on the down-welling limb and requires that the fresh basalt undergoing high-temperature interactions contains 1 p.p.m. boron, which is inconsistent with the very depleted nature of the basalts near the Kane fracture zone³⁴. A more reasonable interpretation is that, like the Cs/Rb ratio, the boron data reflect reaction of high-temperature hydrothermal fluids with basalt that has previously undergone some degree of low-temperature weathering. Although it is not possible to specify a unique boron concentration and isotope composition of the basalt undergoing high-temperature hydrothermal alteration at the MARK site, it is apparent that the boron systematics are significantly different from those of the EPR.

The chondrite-normalized REE patterns of MARK fluids are similar to patterns reported for EPR fluids³⁵. Both are greatly enriched in the light rare earth elements (LREE) and possess a strikingly large, positive Eu anomaly (Fig. 3). The La concentrations in these fluids are 30–90 times that in sea water; Lu concentrations are a more modest 2–5 times greater than in bottom water. The serial trends in these hydrothermal fluids are remarkably similar to phynocryst/matrix partition coefficients for plagioclase minerals³⁶ and leaching of these minerals probably determines the overall pattern.

Although more data is required before any detailed analysis can be attempted, some pertinent observations can be made by examining the normalized lanthanum-to-samarium ratios (La_N/Sm_N) and the europium anomalies ($\text{Eu}^* = \log(2\text{Eu}/\text{Sm} + \text{Gd})$) of the fluids in Table 1. The lower Eu^* of MARK fluids compared with OBS 21° N EPR fluids is consistent with the greater interaction of weathered basalt at the MARK site relative to 21° N EPR. The lower La_N/Sm_N ratios at MARK are also consistent with a depleted magma source along this section of the MAR. In Fig. 3 the greater LREE concentrations in the MARK fluids relative to OBS reflects the higher plagioclase content of Atlantic MORB compared to Pacific MORB³⁷.

The end-member oxygen isotope values for the MARK fluids (Table 1) are significantly heavier than other systems. Extrapolation of the $\delta^{18}\text{O}$ data for the MARK fluids (+2.37‰) is presented in Fig. 4 along with data from other hydrothermal systems. The end-member values for 21° N EPR range from +1.6 to 2.0‰ (refs 31, 38). The range in end-members for the EPR at 13° N and the Juan de Fuca Ridge are between +0.37 and +0.8‰ (refs 39, 40). As the heavier $\delta^{18}\text{O}$ values for MARK may

be a consequence of both variations in the water/rock ratio and the amount of weathered basalt interaction with the fluid, the systematics are unconstrained.

The end-member sulphur isotope value at MARK (+5‰, Table 1; W. C. Shanks III, personal communication), like oxygen, is heavier than most of the 21° N EPR, 11–13° N EPR and S. Juan de Fuca vent fluids (+1 to +5‰) (refs 39, 41). This may be a consequence of anhydrite sulphate-reduction reactions in the mound plumbing system. Woodruff and Shanks⁴¹ proposed a similar mechanism to explain the relatively heavy $\delta^{34}\text{S}$ values for the South West vent field at 21° N EPR.

Conclusions

The first fluid samples collected from a slow-spreading ridge have extended our understanding about the processes that control the chemistry of seafloor hydrothermal systems. Hydrothermal water samples from the TAG and MARK sites along the MAR are remarkably similar to those from the vent sites along the much shallower and faster-spreading EPR. The major element chemistry for the MARK fluids is similar to the vents at 21° N EPR, while the chemistry of the TAG fluids is more similar to the vents near 13° N EPR. The exit temperatures of the MAR hot springs are also similar to the systems along the EPR. Thermodynamic modelling of the MARK solutions indicates that, as with the previously modelled systems of the EPR, these fluids are in equilibrium with respect to a greenschist assemblage of minerals. The $^{87}/^{86}\text{Sr}$ values of the TAG and MARK waters are essentially identical to fresh MORB. But Cs/Rb ratios, boron isotopes and the REE suggest that the MARK fluids have reacted with previously weathered basalt. It may be that the more highly fractured MAR system allows for more interaction of down-welling fluids with previously altered basalts than occurs along faster-spreading systems. The oxygen isotope composition of the end-member MARK fluids is +2.37‰, significantly higher than for the EPR and Juan de Fuca systems. Water-rock ratios cannot be calculated solely from the ^{18}O data for the MARK system because of the unknown magnitude of the weathered basalt component. At this juncture it is not clear to what extent the different tectonic setting of the MAR compared to the EPR contributes to the weathered basalt component seen in the MARK fluids. It appears that the slower spreading rate and greater hydrostatic pressure of the MAR do not, in themselves, produce significant changes in the fluid temperature and major-element chemistry relative to the shallower and faster-spreading EPR.

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Function of *c-mos* proto-oncogene product in meiotic maturation in *Xenopus* oocytes

Noriyuki Sagata*, Marianne Oskarsson*, Terry Copeland*, John Brumbaugh† & George F. Vande Woude*

* Bionetics Research Inc., National Cancer Institute, PO Box B, Frederick, Maryland 21701, USA

† School of Biological Sciences, University of Nebraska, Lincoln, Nebraska 68588, USA

The c-mos proto-oncogene is expressed as a maternal mRNA in oocytes and early embryos of Xenopus laevis, but its translation product pp39^{mos} is detectable only during progesterone-induced oocyte maturation. Microinjection of mos-specific antisense oligonucleotides into oocytes not only prevents expression of pp39^{mos}, but also blocks germinal vesicle breakdown, indicating that it functions during reinitiation of meiotic division.

THE cellular homologue of the viral *mos* (*v-mos*) oncogene of Moloney murine sarcoma virus has been isolated from several vertebrates¹⁻⁵, including human, mouse and chicken. In all these species, the proto-oncogene genomic locus (*c-mos*) contains only a single coding exon. Nucleotide and amino-acid sequence comparisons show that *c-mos* is less well conserved between species^{3,4} than are other members of the *src* kinase family⁶. *c-mos* RNA is expressed at low levels in some adult tissues and in whole embryos of mammals and birds^{3,7}, but in all species the highest levels of *c-mos* expression are detected in gonadal tissues^{3,4,7}. This expression is confined to male and female germ cells⁸⁻¹¹, suggesting that *c-mos* may function during gametogenesis and/or early embryogenesis. Although *c-mos* RNA has been detected, the *c-mos* protein product has so far not been described.

The high degree of *c-mos* expression in oocytes facilitates the study of normal *c-mos* function. One of the best systems is provided by the amphibian *Xenopus laevis*, from which large numbers of oocytes and embryos at specific stages are readily available^{12,13}. Gene expression in the developing oocytes and embryos of this species can also be easily manipulated¹⁴⁻¹⁶. In this report, we provide the first evidence for *mos* proto-oncogene function by demonstrating that *mos*-specific antisense oligonucleotides microinjected into oocytes inhibit meiotic maturation.

cDNA and genomic *Xenopus c-mos*

A complementary DNA library prepared from *Xenopus* oocyte RNA¹⁷ (4.5×10^5 λ gt10 recombinant phages) yielded 11 clones that were positive with both human and chicken *c-mos* probes. The largest cDNA (pXmos-8) was 2.9 kilobases (kb) long and was used to obtain a genomic clone (pXmR-1) from a partial *Xenopus* genomic library (Fig. 1a). Comparison of the cDNA and the genomic clones by extensive restriction enzyme analyses revealed that they were colinear (Fig. 1a).

We determined the nucleotide sequence of the 2.9-kb pXmos-8 cDNA (Fig. 1b). A poly(A) stretch (13 A residues) preceded by a polyadenylation signal, AATAAA, suggested that this clone was complete at the 3' end (position 3,010-3,030; Fig. 1b). By comparison with the genomic locus, we found that an initiator ATG plus 180 additional nucleotides were deleted in pXmos-8 (Fig. 1b) (all other cDNA terminated in the same G-C-rich

region; data not shown). Collectively, it was found that the *Xenopus c-mos* (*c-mos^{xc}*) open reading frame is 1,080 nucleotides long and is only 52-55% homologous with the human, mouse, or chicken *c-mos* loci in nucleotide sequence. The *c-mos^{xc}* coding region is G-C rich (overall 65%), and the number of CpG dinucleotides, which is normally depressed in the vertebrate genome¹⁸, is close to the non-depressed CpG level (85%). The nucleotide sequence also indicates that the *c-mos^{xc}* RNA contains a very long (2 kb) 3' untranslated region (Fig. 1b), which is unusual when compared with mammalian and some avian *c-mos* transcripts^{3,4,8}. In this 3' region, we note the presence of five ATTTA motifs that could influence the stability of messenger RNA (ref. 19). These studies represent the first characterization of a *c-mos* cDNA.

The *c-mos^{xc}* open reading frame consists of 359 amino acids that would yield a protein of relative molecular mass (M_r) ~ 39,000 (39K). The amino-acid sequence homologies of *c-mos^{xc}* compared with human, mouse and chicken are 53, 51 and 56% respectively. Some of the conserved domains show partial homology with other members of the *src* kinase family (closed circles, Fig. 1c), but several are *mos*-specific. The *c-mos^{xc}* has an additional 20 amino acids at its C-terminus, which is proline and glycine-rich (Fig. 1c).

c-mos^{xc} RNA in adult tissues

Total RNA from adult *Xenopus* tissues was subjected to Northern analysis using a *c-mos^{xc}* probe. Only three tissues were positive by this assay (Fig. 2a). Very low concentrations of *c-mos* transcripts were detected in brain and testes, but a high level was observed in ovaries. Expression of *c-mos* in *Xenopus* brain and gonadal tissues is similar to the expression pattern observed in mammals and birds^{3,4,8}. The size of *c-mos^{xc}* transcripts (3.1 kb) was consistent with the size and structure of the pXmos-8 cDNA (Fig. 1a and b) and was identical in all three tissues. This is in contrast to the tissue-specific *c-mos* transcript size differences observed in mammals^{4,7}.

To determine the transcription start site of the ovarian *c-mos^{xc}* messenger RNA, we performed S1-nuclease mapping analysis using a 348-base end-labelled *Pst*I-AccI fragment, which spans the initiator ATG codon (Fig. 1a), as probe. Two major and two minor fragments were protected from S1 nuclease digestion

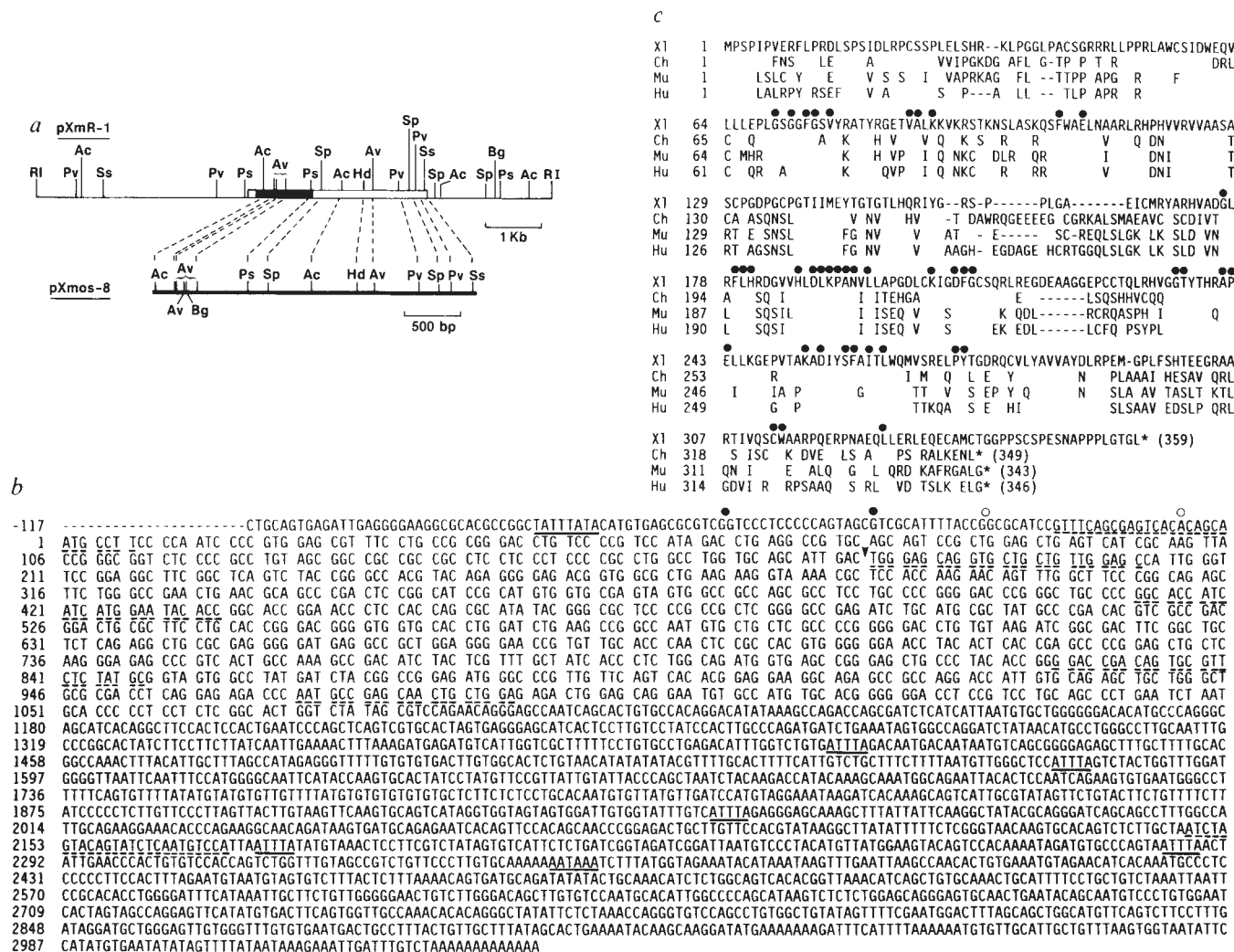


Fig. 1 Restriction map comparison of the genomic and cDNA clones of *Xenopus c-mos* (a), nucleotide sequence of *Xenopus c-mos* cDNA (b) and amino-acid sequence comparison of vertebrate *c-mos* genes (c). a, Restriction maps of the genomic (pXmR-1) and cDNA (pXmos-8) inserts are compared. The closed box in pXmR-1 represents the *c-mos* coding region, and open boxes correspond to the 5' and 3' untranslated regions. The vector-derived *EcoRI* sites¹⁷ at both ends of the pXmos-8 cDNA and several *PstI* sites upstream of the *c-mos* locus in pXmR-1 are omitted. An *AvaI* and a *BglII* site (shown below the line in pXmos-8) are absent in pXmR-1 and represent restriction site polymorphisms (data not shown). Ac, *AccI*; Av, *AvaI*; Bg, *BglII*; Hd, *HindIII*; Ps, *PstI*; Pv, *PvuII*; RI, *EcoRI*; Sp, *SpeI*; Ss, *SstI*. b, Nucleotide sequences of the entire pXmos-8 cDNA and a portion of the pXmR-1 genomic clone. The sequence from positions 178 (arrowhead) to 3,043 was derived from pXmos-8. Sequences upstream and overlapping pXmos-8 were derived from pXmR-1. The *c-mos* coding frame is represented by triplet codons, starting at position 1 and ending at position 1,080. TATA box-like sequence (TATTTATA), a polyadenylation signal (AATAAA) and mRNA-stabilizing ATTTA motifs¹⁹ are underlined. Two major and two minor candidate transcription start sites are indicated by closed and open circles respectively. Nucleotide sequences used as sources of synthetic sense and antisense oligonucleotides are underlined (dashed line; see Table 1). c, Complete amino-acid sequence of *c-mos*^{xc} (X1) deduced from the nucleotide sequence (b) is aligned with the chicken (Ch) (ref. 3), mouse (Mu) (ref. 1) and human (Hu) (ref. 2) *c-mos* amino-acid sequences. The amino-acid sequence for chicken, mouse or human is included only when it differs from *c-mos*^{xc}. Among the amino acids in common, those which are conserved among members of the *src* kinase family¹⁶ are identified by closed circles.

Methods. A *Xenopus* oocyte cDNA library (4.5×10^5 phages) in λ gt10 (ref. 17) was screened with ³²P-nick-translated human² and chicken³ *c-mos* probes. Prehybridization and hybridization were as described²⁵ but at lower stringency (20% formamide). The hybridized filters were washed at 50 °C for 4 h with several changes of 1 × SSC (0.15 M NaCl and 0.015 M sodium citrate) containing 0.2% SDS. This screening gave eleven clones, from pXmos-1 to pXmos-11. Southern transfer analysis of the *Xenopus* genomic DNA using pXmos-8 cDNA (insert) as probe showed a single 9.1 kb *EcoRI* fragment. This DNA fraction was isolated and used to construct a partial genomic library in λ gtWES- λ B phage vector, which gave seven *c-mos* genomic clones on screening, from pXmR-1 through to pXmR-7. Insert DNA fragments of both the cDNA (pXmos-8) and genomic (pXmR-1) clones were subcloned into pUC18 plasmids and restriction maps were constructed by double and triple digestion methods. Nucleotide sequence was determined by an automated chain-termination method using primers with multiple fluorophores^{56,57}.

(Fig. 2b), indicating possible transcription initiation sites located respectively at -60, -45, -30 and -5 base pairs (bp) upstream from the initiator ATG at position 1 (Fig. 1b). Multiple transcription initiation sites have also been observed in mammalian *c-mos* transcripts^{4,8}. Unlike mammalian and avian *c-mos* loci^{2,4,8}, the *c-mos*^{xc} genomic locus has a TATA-like sequence at -80 bp upstream from the initiator ATG (Fig. 1b). Similarly sized DNA

fragments were also protected in S1 analyses of *Xenopus* testicular transcripts (data not shown), indicating that, unlike in mammals^{4,8}, common promoters are apparently used for amphibian *c-mos* transcripts in both ovaries and testes. These data, plus restriction map comparison of the cDNA versus the genomic locus (Fig. 1a), indicate that *c-mos*^{xc} RNA transcripts, like those in mammals^{4,8}, are not processed.

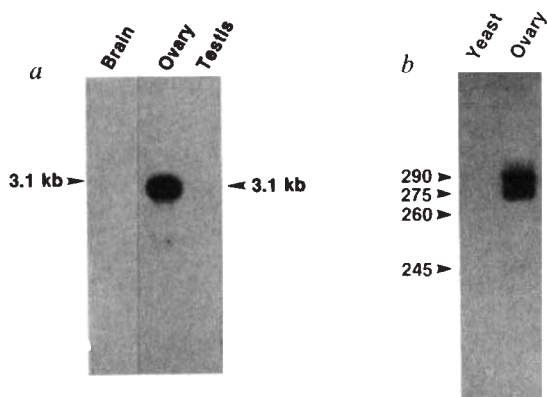


Fig. 2 *c-mos^{xc}* RNA expression in adult *Xenopus* tissues (a) and S1-mapping analysis of transcription start sites (b).

Methods. a, Total RNA was extracted from ~20 adult *Xenopus* tissues (brain, eye, skin, lung, liver, stomach, intestine, pancreas, spleen, adrenal, heart, kidney, muscle, oviduct, ovary and testes), as described⁵⁸. Total RNA (30 µg) was electrophoresed in a 1.2% agarose gel containing 2.2 M formaldehyde, and analysed by Northern transfer⁵⁸ using ³²P-nick-translated pXmos-8 cDNA (insert) as probe. Only positive tissues are shown. The faint 2-kb band in the ovary is possibly an artefact, but was not further characterized. b, Total RNA (25 µg) from ovaries (yeast RNA was used as a negative control) was subjected to S1-mapping analysis using a *Pst*I-AccI fragment (348 bases long) corresponding to the 5' end of the *c-mos^{xc}* RNA as probe (Fig. 1a). This probe was ³²P-end-labelled at the AccI site by kination⁵⁸; hybridization was performed at 60 °C as previously described⁷. The S1-protected sizes are indicated in bases.

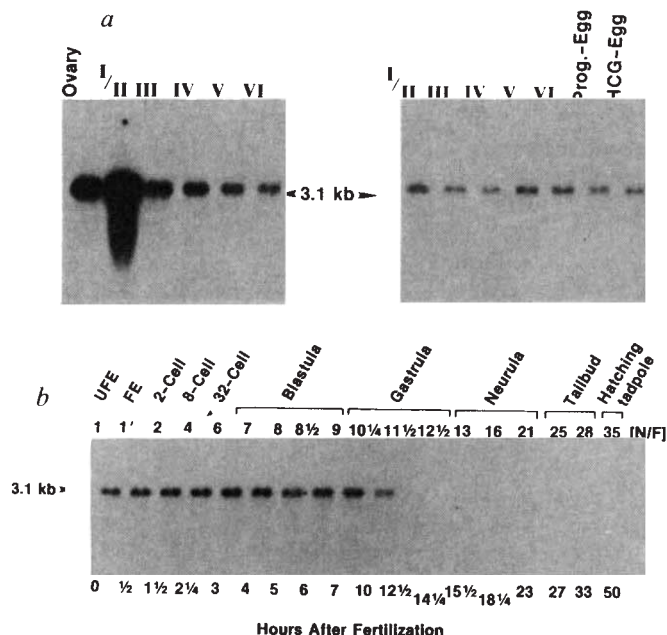


Fig. 3 *c-mos^{xc}* mRNA expression during oogenesis (a) and embryogenesis (b).

Methods. Ovaries were manually dissected and treated with collagenase (2 mg ml⁻¹) in modified Barth solution (MBS) to obtain defolliculated oocytes¹⁴. The oocytes (from I to VI) were staged according to Dumont¹². Eggs matured *in vitro* were obtained by the treatment of stage VI oocytes with progesterone (5 µg ml⁻¹) for 12 h. Eggs and embryos were obtained from HCG-injected animals as described²³, developed at 20–22 °C in 10% MBS and staged according to Nieuwkoop and Faber¹³. They were dejellied with 2% cysteine before use¹⁴. RNA extracted from the samples was subjected to Northern transfer analysis (see Fig. 2) using ³²P-labelled nick-translated pXmos-8 as probe.

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to mature *in vitro* with progesterone (a physiological inducer of amphibian oocyte maturation²⁵) were metabolically labelled and subjected to immunoprecipitation analyses using the C237 antiserum (Fig. 4a). None of the polypeptides precipitated in extracts of small (stages I and II), medium (stages III and IV) or fully grown (stages V and VI) oocytes appeared to be specifically recognized (that is, blocked in the presence of the competing peptide). In contrast, two polypeptides (45K and 39K) were specifically precipitated by the C237 antiserum from oocytes induced to mature with progesterone (arrows, lane MO, Fig. 4a). Using the same antiserum, immunoprecipitation analysis of embryos during blastula and gastrula stages identified several polypeptides (arrows lanes B and G, Fig. 4a). No candidate proteins were identified in neurula stage embryos (lane N, Fig. 4a). Although we identified several polypeptides with the C237 antiserum during different stages of development, only the 39K protein (p39) found specifically in maturing oocytes was also recognized by the other two *c-mos^{xc}* specific antisera (B-mos, C232; Fig. 4b). Thus both the *M_r* of protein p39 and its recognition by three different *mos*-specific antisera are consistent with its being the *c-mos^{xc}* proto-oncogene product.

The band width of the p39 product suggested that it was post-translationally modified. To determine whether it is phosphorylated like the p37^{v-mos} product²⁶, immature and maturing oocytes were labelled with carrier-free ³²P and immunoprecipi-

c-mos^{xc} is expressed as maternal mRNA

c-mos^{xc} RNA expression in ovaries is restricted to oocytes (data not shown). We examined *c-mos^{xc}* RNA expression in growing oocytes from stage I (smallest, 50–150 µm in diameter, 100 ng RNA per oocyte) to stage VI (fully grown, 1.2 mm in diameter, 5 µg RNA per oocyte) according to Dumont¹². Northern analysis of a fixed amount of total RNA (30 µg) revealed that the highest level of *mos* transcripts was present in the smallest oocytes (stages I and II). The level was substantially lower in fully grown stage VI oocytes (5% of stage I and II; left panel in Fig. 3a). But on a per oocyte basis, the amount of *c-mos^{xc}* RNA was essentially constant during growth, as well as during oocyte maturation *in vitro* or *in vivo* (right panel in Fig. 3a). Moreover, total RNA (30 µg) from different developmental stages after fertilization showed that the same level of *c-mos^{xc}* RNA persisted even through cleavage (stages 3–6, according to Nieuwkoop and Faber¹³) and blastulation (stages 7–9) (Fig. 3b). But at the same time as gastrulation (stages 10–12½), *c-mos^{xc}* RNA disappeared (half life 2.5 h; data not shown) and was not detected in subsequent stages (Fig. 3b). As virtually no RNA synthesis or major changes in total RNA content occur between the fertilization and blastula stages^{20–22}, it is likely that *mos* RNA present during early oogenesis persists through blastulation as a maternal mRNA^{20,23}. We do not observe specificity in the localization of *c-mos^{xc}* maternal mRNA in embryonic regions of cleavage and blastula embryos (data not shown).

Identification of the *mos* protein product

Although *mos* mRNA is present throughout oogenesis and early embryogenesis, it is possible that the protein product is only expressed at a specific stage or stages during development^{20,24}. We raised antisera against a portion of the *c-mos^{xc}* gene product expressed in bacteria (B-mos antiserum) and against two synthetic peptides from the C-terminal region of the open reading frame (C232 and C237 antisera; Fig. 4). These three antisera specifically recognized the bacterially expressed *c-mos^{xc}* protein product (data not shown). Growing oocytes and oocytes induced

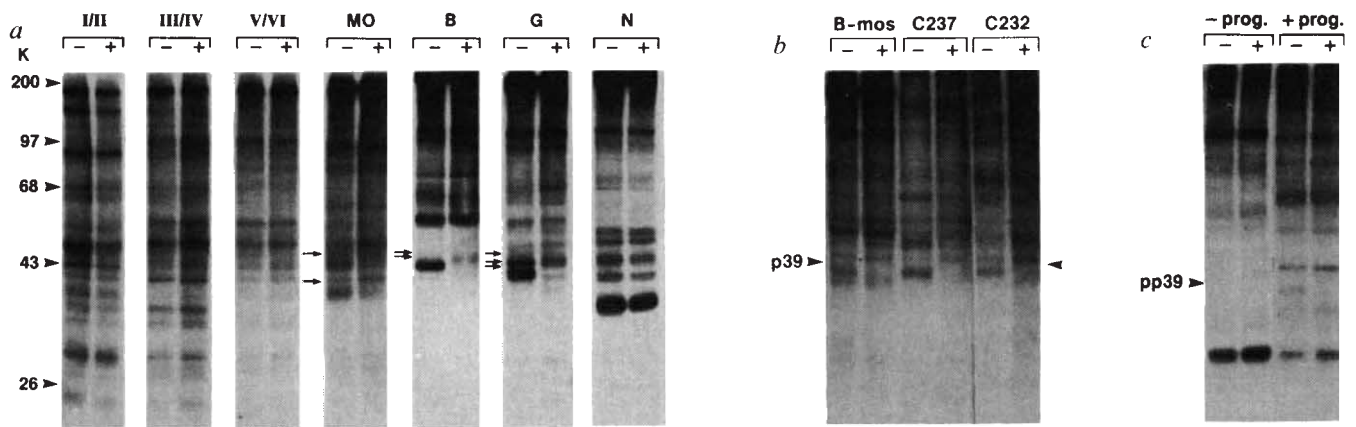


Fig. 4 Immunoprecipitation analysis of *c-mos^{sc}* product. *a*, Immunoprecipitation of oocyte and embryo extracts with C237 antiserum in the presence (+) or absence (-) of competing peptide antigen. Oocytes and maturing oocytes were metabolically labelled with both [³⁵S]methionine and [³⁵S]cysteine for 11 h, and embryos were labelled for 5 h. In all samples, $\sim 1 \times 10^7$ c.p.m. were subjected to immunoprecipitation. Arrows point to polypeptides whose immunoprecipitation was blocked by the competing antigen. I/II, a pool of stage I and II oocytes; III/IV, a pool of stage III and IV oocytes; V/VI, a pool of stage V and VI oocytes; MO, maturing oocytes treated with progesterone; B, blastula embryo (metabolically labelled from stage 7½ to stage 10); G, gastrula embryo (stage 10 to stage 12); N, neurula embryo (stage 15 to stage 18). *b*, Immunoprecipitation of maturing oocyte extracts with three different antisera (B-mos, C232, C237) in the presence (+) or absence (-) of corresponding competing antigen. Maturing oocytes treated with progesterone were labelled with both [³⁵S]methionine and [³⁵S]cysteine for 11 h. *c*, Immunoprecipitation of immature (-prog.) or maturing (+prog.) oocyte extracts with C237 antiserum in the presence (+) or absence (-) of the competing antigen. Stage VI oocytes preloaded with carrier-free ³²P were cultured for 11 h with or without progesterone. **Methods.** Oocytes and embryos were prepared as described in Fig. 3 legend, except that collagenase treatment of oocytes was for 1–2 h. Oocytes and maturing oocytes were cultured at 19–21 °C in MBS containing both [³⁵S]methionine (1 mCi ml⁻¹) and [³⁵S]cysteine (1 mCi ml⁻¹) for the period specified. For the maturing oocytes, fully grown stage VI oocytes were treated with progesterone (5 µg ml⁻¹) simultaneously with isotopic labelling. For ³²P labelling, stage VI oocytes were preloaded with carrier-free ³²P (5 mCi ml⁻¹) for 4 h and then cultured for 11 h in fresh MBS with or without progesterone. Embryos were dissected into halves in MBS to facilitate incorporation of exogenously added isotopes ([³⁵S]methionine and [³⁵S]cysteine, each at 1 mCi ml⁻¹)⁵⁹ and cultured at 21–23 °C for the specified period. Microinjection of the same isotopes into intact embryos yielded similar results (data not shown). Peptide antisera, C232 and C237, were raised in rabbits against synthetic peptides, AEQLLERLEQECAM and CTGGPPSPESNAPPLGTGL respectively, both of which were derived from the C-terminal region of the *c-mos^{sc}* protein (Fig. 1c). Another antiserum, B-mos, was raised against a bacterially expressed λ CII-*mos^{sc}* fusion protein produced by the vector pAJ π (ref. 60) into which a *Bgl*II-*Rsa*I fragment from pXmos-8 cDNA (Fig. 1b) was inserted. All procedures for immunoprecipitation and acrylamide gel electrophoresis were as described⁶¹.

tated with C237 antiserum (Fig. 4c). These analyses showed a 39K phosphoprotein only in oocytes induced to mature with progesterone. This product was also detected with C232 antiserum (data not shown). Thus the 39K protein appears to be phosphorylated (pp39).

Inhibition of pp39 synthesis

Recently it has been shown that microinjection of antisense oligodeoxynucleotides (oligonucleotides) into *Xenopus* oocytes leads to degradation of both exogenous and endogenous mRNA species and prevents synthesis of specific protein products^{27–29}. To test whether *mos* oligonucleotides alter levels of the endogenous *mos* maternal mRNA, we microinjected a mixture of either four antisense oligonucleotides or the corresponding four sense oligonucleotides, into fully grown oocytes. RNA extracted at 1, 4, and 14 hours postinjection was analysed by dot blot analysis for the presence of *mos* mRNA (Fig. 5a). Only oocytes injected with *mos* antisense oligonucleotides showed a significant reduction in *mos* RNA in 4 h and nearly complete loss in 14 h. Therefore, only these oocytes should show a reduction in pp39 after progesterone treatment if it is the authentic *mos* product.

We injected a mixture of either four antisense or four sense oligonucleotides into fully grown oocytes as above. After 4 h, injected and control oocytes were both stimulated to mature with progesterone, metabolically labelled continuously for 11 h, and the extracts immunoprecipitated using C232 antiserum (Fig. 5b). We detected pp39 in oocytes microinjected with the mixture of sense oligonucleotides (Fig. 5b), as well as in control oocytes (not shown, compare Fig. 4b), but not in oocytes microinjected with the mixture of antisense oligonucleotides. Similar results were obtained using a C237 antiserum (data not shown). These

results provide evidence that pp39 is the *mos*-specific translation product (pp39^{*mos*}).

Prevention of oocyte maturation

In the above experiments (Fig. 5b), we observed that oocytes injected with *mos* antisense oligonucleotides failed to undergo germinal vesicle breakdown (GVBD) after treatment with progesterone, whereas control oocytes and those injected with the sense oligonucleotides matured normally (Fig. 5c). To substantiate this finding we examined the effect of individual oligonucleotides on oocyte maturation (Table 1). More than 95% of the oocytes microinjected with sense oligonucleotides, either as a mixture or individually, matured normally when compared with control progesterone-treated oocytes. In contrast, all *mos* antisense oligonucleotides markedly inhibited oocyte maturation when microinjected as mixtures (~85%) or when individually injected (~76%) at concentrations of 2–4 mg ml⁻¹ (Table 1). Of the six oligonucleotides injected individually, five were complementary to sequences in the coding region, and one (D⁻) was in the 3' untranslated region. Inhibition of GVBD was concentration-dependent.

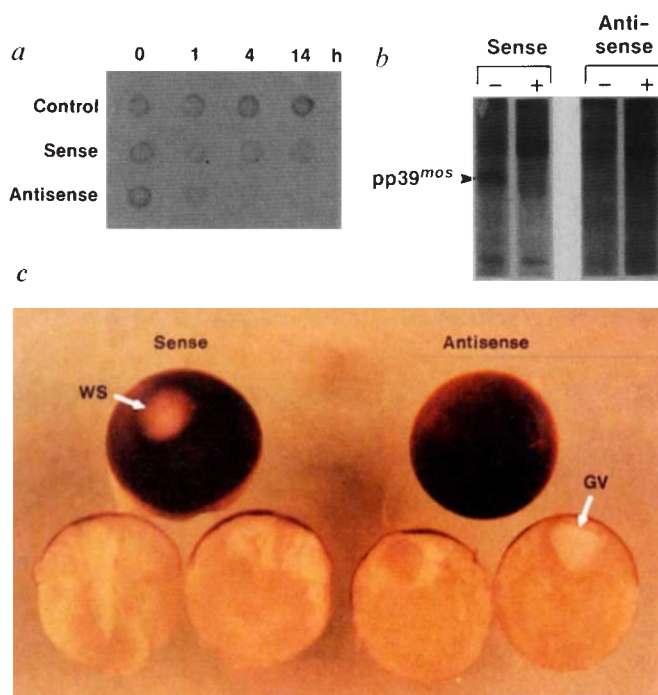
We also examined the effect of either sense or antisense oligonucleotides on oocyte maturation induced by insulin, a potent *in vitro* inducer of *Xenopus* oocyte maturation³⁰. Again, GVBD was markedly inhibited by the *mos* antisense oligonucleotides (Table 1). Collectively, these results demonstrate that the *mos* proto-oncogene product, pp39^{*mos*}, is essential for meiotic maturation.

Translational regulation of *c-mos^{sc}* mRNA

Despite the persistence of *c-mos^{sc}* RNA throughout oogenesis and early embryogenesis, pp39^{*mos*} synthesis is detectable only

Fig. 5 Effects of *mos* antisense oligonucleotides on the level of endogenous *c-mos*^{xe} mRNA (a), the synthesis of pp39^{mos} (b), and the morphology (c) in microinjected oocytes.

Methods. Stage VI oocytes prepared as in Fig. 4 were mock-injected (control) or microinjected with 65 nl solution (88 mM NaCl, 15 mM Tris-HCl, pH 7.5) containing a mixture of either four sense (A⁺, B⁺, C⁺, D⁺, specified in the legend to Table 1) or the corresponding four antisense (A⁻, B⁻, C⁻, D⁻, Table 1) oligonucleotides (1 mg ml⁻¹ for each oligonucleotide). *a*, Total RNA (15 µg) extracted at 0, 1, 4 and 11 h postinjection was subjected to dot-blot analysis⁵⁵ using pXmos-8 as probe. *b*, Oocytes were microinjected as above. Four hours later, they were treated with progesterone and labelled with both [³⁵S]methionine (1 mCi ml⁻¹) and [³⁵S]cysteine (1 mCi ml⁻¹) for 10 h as described in Fig. 4 and immunoprecipitation analysis was performed using C232 antiserum in the presence (+) or absence (-) of the peptide antigen. *c*, Four hours after microinjection, oocytes were treated with progesterone. After 12 h they were fixed with 5% TCA for 30 min, manually dissected with fine needles, and observed under a stereomicroscope. Oocytes injected with sense oligonucleotides matured normally and displayed a white spot (WS) indicative of GVBD (ref. 62), and those injected with *mos* antisense oligonucleotides retained the germinal vesicle (GV) intact.



during progesterone-induced oocyte maturation. During this period, total protein synthesis increases twofold but little, if any, change in the pattern of polypeptides synthesized is observed^{31,32}. Our results indicate that pp39^{mos} synthesis is uniquely stimulated by progesterone. The precise mechanism of regulation is unknown, but it is probably indirect as the steroid hormone acts firstly on the surface membrane of the oocyte to cause very early maturation events, and then the increase in protein synthesis occurs later³³.

In preliminary experiments, pp39^{mos} synthesis is not detected between the 2-cell and late cleavage (stage 7) stages (data not shown) or during blastulation (Fig. 4a). During these periods, the bulk of maternal mRNA is recruited on to polysomes and supports a several-fold increase in total protein synthesis²⁰. Thus, the translational regulation of maternal *c-mos*^{xe} RNA after fertilization is also novel and differs markedly from other maternal mRNA species. It is possible that *mos* mRNA merely persists until gastrulation, when general degradation of maternal mRNA occurs^{20,23}. Alternatively, undetectably low levels of pp39^{mos} might still be expressed during these embryonic stages.

pp39^{mos} functions during meiotic maturation

Using *mos*-specific antisense oligonucleotides, we showed inhibition of GVBD in progesterone-treated oocytes; all six *mos* antisense oligonucleotides showed significant GVBD inhibition, whereas the five sense oligonucleotide did not. These data strongly argue that the *mos* product is required for oocyte maturation and not only provide the first evidence for *mos* proto-oncogene function, but also demonstrate that meiotic maturation can be inhibited by antisense oligonucleotides. This is in contrast to the recent results reported for *Xenopus* β -tubulin, where antisense oligonucleotides also blocked *de novo* synthesis of the protein but failed to prevent oocyte maturation, presumably because of pre-existing β -tubulin pools in immature oocytes²⁹.

In most species, oocyte maturation begins with a fully grown oocyte arrested at meiotic prophase I, proceeds through a series of well characterized events, such as GVBD, chromosome condensation, spindle formation and cytokinesis, and ends with a fully mature oocyte (egg) arrested at meiotic metaphase II (ref. 25). In *Xenopus*, oocyte maturation usually takes 8–12 h (both *in vivo* and *in vitro*) and GVBD occurs 4–6 h after progesterone treatment *in vitro*²⁵. It has been established that protein synthesis

induced by progesterone is a prerequisite for GVBD (refs 34 and 35). The inhibition of GVBD by *mos* antisense oligonucleotides implies that the synthesis of *mos* protein both precedes and is required for GVBD. Thus, its *de novo* expression during this period can explain at least in part the protein synthesis requirement for GVBD. In terms of the cell cycle, a fully grown oocyte is arrested at the G2/M border and GVBD occurs shortly before M phase³⁵. It follows that the *mos* protein may function during the transition from G2 to M in the first meiotic division.

Maturation or M-phase promoting factor (MPF) appears in *Xenopus* oocytes shortly before GVBD (refs 25 and 33). MPF, first discovered in unfertilized eggs³⁶ and now known to occur in somatic cells^{33,35}, is believed to directly cause GVBD and chromosome condensation^{25,34}. It is generally agreed that MPF is a protein kinase(s) with autocatalytic activity^{33,35,37}, and its appearance is tightly coupled with protein phosphorylation^{33,38}, cycling and peaking at metaphase in both the first and second meiotic divisions, as well as in mitotic division^{35,39,40}. Recently MPF has been purified nearly to homogeneity and shown to consist of a complex of two proteins⁴¹. One of the components has been shown to be the homologue of the fission yeast *S. pombe cdc2* gene product, p34, a serine kinase that controls entry into mitosis^{42,43}. Therefore this function is conserved in eukaryotes^{42–44}. In amphibians the appearance of MPF activity before GVBD requires protein synthesis, but MPF itself can induce GVBD independently of protein synthesis^{33,39,41}. Furthermore, MPF pre-exists in immature oocytes as an inactive precursor (pre-MPF) and its activation appears to occur through phosphorylation^{39,45}. These results suggest the existence of an unknown protein(s) that is synthesized *de novo* before the appearance of MPF activity and leads to activation of pre-MPF by phosphorylation⁴⁵. As a putative serine/threonine protein kinase^{6,46}, pp39^{mos} could be this protein and function directly or indirectly upstream of MPF to activate pre-MPF, but we cannot exclude that it may be a component of MPF. We note that invertebrate cyclins may also activate MPF, but the two which have been sequenced show no homology with the *mos* product^{47,48}. Cyclin protein species are synthesized in abundance during interphase and are rapidly degraded after metaphase⁴⁷. We have not determined whether pp39^{mos} cycles in a similar fashion and it is not clear how the *mos* product could participate in a pathway with cyclins.

The *mos* protein may also function after GVBD during the

Table 1 Effect of *mos*-specific oligonucleotides on GVBD after treatment with progesterone or insulin

Oligo-nucleotide*	Oligonucleotide position	Length in nucleotides	Concentration (mg ml ⁻¹)	Number of experiments	Total number of injected oocytes	Total number of oocytes with GVBD	% GVBD inhibition
Control							
None	—	—	—	6 (2)†	111 (38)	110 (36)	1 (5)
Buffer	—	—	—	4	82	81	1
Sense							
Mixture (A ⁺ ~ D ⁺)	coding/noncoding	24-25	4	6 (2)	123 (41)	117 (38)	5 (8)
A ⁺	ATG-spanning	25	2	5	118	113	4
B ⁺	mid-coding	24	2	4	92	89	3
C ⁺	3'-coding	24	2	3	72	69	4
D ⁺	3'-noncoding	25	2	3	78	75	4
E ⁺	3'-coding	20	2	4	81	79	3
				2	49	46	6
Antisense							
Mixture (A ⁻ ~ D ⁻)	coding/noncoding	24-25	4	6 (3)	149 (63)	18 (12)	88 (81)
A ⁻	ATG-spanning	25	2	5	125	23	82
			4	3	83	17	80
			2	4	91	24	74
			1	2	54	19	65
B ⁻	mid-coding	24	1	3	71	30	58
			0.25	2	48	38	21
C ⁻	3'-coding	24	1	3	79	35	56
D ⁻	3'-noncoding	25	4	4	95	21	78
			2	4	100	27	73
			1	3	75	27	64
F ⁻	ATG-spanning	25	2	3	81	25	69
G ⁻	5'-coding	25	2	3	78	13	84

Fully grown stage VI oocytes were prepared as described in Fig. 3 legend, except that oocytes to be exposed to insulin were not treated with collagenase. Oocytes were then microinjected with the indicated oligonucleotides, either as a mixture or individually at the indicated concentrations. Four hours after injection, oocytes were treated either with progesterone (5 µg ml⁻¹) for 12–14 h or with insulin (35 µg ml⁻¹) for 16–18 h, and fixed with 5% TCA. Each oocyte was examined for GVBD both externally and internally, as described for Fig. 5c. Oocytes were discarded that spontaneously matured before hormonal treatment, or within 1.5 h after treatment. The percentage GVBD inhibition in oocytes microinjected with antisense oligonucleotides varied with a s.d. of ±15% between batches of oocytes. In total, 15 female frogs that had not been previously injected with human chorionic gonadotrophin were used.

* The nucleotide sequences of the oligonucleotides used correspond to positions in the *c-mos*^{xe} locus (Fig. 1b): A = (–)18–7; B = 412–435; C = 825–848; D = 2,148–2,172; E = 967–986; F = (–)21–4; G = 178–202.

† Numbers in parentheses are for insulin-treated oocytes.

second metaphase when MPF activity reappears^{35,39}; detection of pp39^{mos} in maturing oocytes after 11 h of progesterone treatment (Fig. 4) implies that it is present in eggs arrested at the second metaphase.

Insulin can trigger meiosis in *Xenopus* oocytes *in vitro*³⁰, but this induction is apparently mediated by endogenous *ras* protein although progesterone induction is not⁴⁹. Our results show that both progesterone- and insulin-induced oocyte maturation are inhibited by *mos* antisense oligonucleotides (Table 1). Therefore both pathways (including *ras* function in the insulin pathway) must overlap upstream of *mos* and MPF. Previous studies in transformed cells have indicated either that *ras* and *mos* are in separate pathways, or that *mos* is downstream from *ras*^{50,51}. Our studies provide evidence for the latter.

Does pp39^{mos} function during mitosis?

The *mos* product may be specific for meiosis, but it is possible that it also functions in mitosis after fertilization. Fertilization to mid-blastula stage in *Xenopus* embryos is characterized by 12 rapid, synchronous cell divisions without G1 and G2 phases²². These rapid cell divisions are also associated with the cycling of protein phosphorylation and MPF activity^{35,40}. Although *mos* protein synthesis was not detected after fertilization, it could occur at very low levels (that is, transiently) in cleaving cells. Protein derived from this synthesis or from a pool pre-existing in unfertilized eggs (compare Fig. 4) could contribute to the activation of MPF during the first few hours of development.

Our results suggest that *mos* may act in conjunction with MPF during meiosis and the similarities of events in meiosis and mitosis suggest that similar products play a part in both pro-

cesses^{39,40,45}. Very low levels of *c-mos* expression have been observed in a variety of non-gonadal tissues^{3,4,8} (Fig. 2a) and extremely low levels of *v-mos* RNA and protein products have been shown to function in *v-mos*-transformed cells^{26,52}. These results, together with the high *c-mos*^{xe} CpG content (Fig. 1b) and its hypomethylated state in adult tissues (data not shown), properties of CpG islands¹⁸, raise the possibility that the *c-mos* proto-oncogene may be a 'housekeeping' gene that functions at very low levels in many cell types.

mos as an oncogene

In somatic cells, constitutive expression of *mos* results in transformation⁵³. Thus, *c-mos*^{xe} also transforms mouse NIH 3T3 cells, albeit at low frequency (M. Oskarsson, unpublished data). It is possible that its transforming activities could represent a subset of its normal activity and that the transformed phenotype could be due to activation of some G2-M transition events at the wrong time during the cell cycle. For example, the transformed cell phenotypes of morphological alteration, anchorage independence and loss of contact inhibition could equate with mitotic cell rounding and a phenotype that prevents growth arrest during cytokinesis. Moreover, premature chromatin condensation could promote chromosomal abnormalities. Recently a similar argument has been presented for the explanation of the transformed phenotype caused by the *src* gene product whose threonine/serine phosphorylation and kinase activity is mitotically regulated⁵⁴. This may be a general explanation for how oncogenes influence expression of the transformed phenotype.

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have been submitted to the EMBL/GenBank Libraries.

Note added in proof: Using a *mos*^{xc}-specific monoclonal antibody and a short labelling period, we detect low levels of pp39^{mos} in fully grown oocytes. We also find that injection of synthetic *c-mos*^{xc} RNA induces GVBD in the absence of progesterone.

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LETTERS TO NATURE

Structural ordering in a calcium silicate glass

M. C. Eckersley*, P. H. Gaskell*, A. C. Barnes† & P. Chieux†

* University of Cambridge, Cavendish Laboratory, Madingley Road, Cambridge CB3 0HE, UK

† Institut Laue-Langevin, Avenue des Martyrs, 38042 Grenoble Cedex, France

Glasses, and the liquids from which they are generally quenched, conspicuously lack translational periodicity and from this experimental fact it is a short step to assume that glasses are essentially disordered. In silicate glasses, the strongly covalently bonded silicon and oxygen atoms are thought to form a network structure within which ionically bonded 'network-modifying' atoms such as Li, Na, Ca, Mg and Fe substitute randomly in suitable interstices. Taken to extremes, this view leads to a structure in which any local order is restricted to the environment of the strongly bound Si atoms, with ions like Ca²⁺ having a highly distorted and variable first coordination shell of oxygens. The evidence for local ordering around the Si is overwhelming; in vitreous silica, SiO₄ tetrahedra are only a little more distorted than in the crystal. Here we present conclusive experimental evidence, from neutron scattering with isotopic substitution, that Ca, a typical alkaline-earth 'modifier', also has a comparatively well ordered first-neighbour shell in a silicate glass, with indications of ordering beyond that.

The assumption of prevailing disorder in glasses has met with a number of direct and indirect challenges. One approach to structural studies has been to recognise essential similarities between microscopic and macroscopic properties of glasses and crystals and to interpret these in terms of equivalent structural units. For the simple silicates, comparison of the structure of glasses and crystals is particularly revealing. Dent-Glasser¹ has shown that although silicate minerals exist with a bewildering variety of structural motifs, a stable lattice is often the result of a delicate compromise between packing constraints imposed by the silicon-oxygen framework and the coordination requirements of ions such as Ca²⁺. For a number of compositions, the two sets of constraints prove to be mutually incompatible and structures that are topologically possible are not observed in practice. Crystalline calcium disilicate, CaSi₂O₅, is a relevant example. Furthermore, the structure of, for example, single-chain metal silicate lattices depends on the size and charge of the metal atoms. Liebau² has demonstrated that the number of SiO₄ tetrahedra forming a repeating unit, and their relative disposition, is a sensitive function of these parameters. In this sense, the metal cations exert an important, perhaps even dominant, influence on the structure.

Do the same constraints apply to amorphous silicates? Dent-Glasser suggests that some of them may, and argues that the common occurrence of immiscibility in silicate melts may be a manifestation of the same structural incompatibility¹. Furthermore, Gaskell³ has shown that the similarity of density data for crystalline and amorphous silicates implies a structure-forming role for the 'network-modifying' elements.

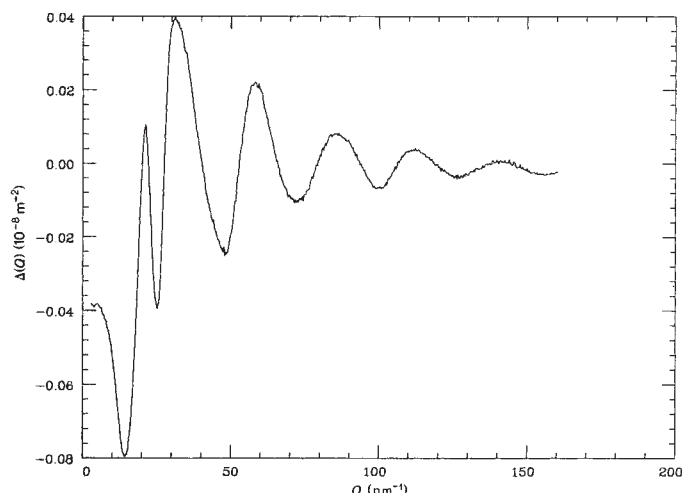


Fig. 1 Experimental difference function, $\Delta(Q)$, obtained from scattering data for two calcium aluminosilicate glasses of approximately metasilicate composition, CaSiO_3 , with isotopic substitution of Ca. $\Delta(Q)$ oscillates around a mean value that departs slightly from zero at high values of Q . This was corrected by fitting $\Delta(Q)$ with a second-order polynomial and treating this as the baseline, before Fourier transformation to give $G_{\text{Ca}}(r)$ (Fig. 2).

Further progress depends on direct measurements of the environment of atoms such as Ca in amorphous silicates. If Ca exerts a significant influence on its immediate environment, this should be recognizable as a well defined first-neighbour shell; specifically, the coordination number and the mean and standard deviation of the Ca–O distribution would be then close to the values found in the most stable phase—the compositionally equivalent crystal. Indications of order in higher-neighbour shells would, of course, provide supportive evidence.

Some evidence from extended X-ray absorption fine structure (EXAFS) data for sodium silicate and calcium aluminosilicate glasses has been presented by Greaves *et al.*^{4,5}, Geere *et al.*⁶ and Brown *et al.*⁷. There is by no means a consensus but the weight of evidence favours a relatively well defined first coordination shell for Na and Ca, with a suggestion that the Ca environment may be reasonably insensitive to composition.

We have investigated the structure around Ca in a silicate glass, lightly doped with Al_2O_3 to prevent crystallization. Two specimens of composition $(\text{CaO})_{48.0}(\text{SiO}_2)_{49.0}(\text{Al}_2\text{O}_3)_{3.0}$ were prepared, which were identical in all respects except for the isotopic composition of Ca: one glass contained natural Ca and the other contained essentially pure ^{44}Ca . A composition close to the metasilicate was chosen to enable a direct comparison with *c*- CaSiO_3 , after an extensive series of tests to minimize problems due to immiscibility and crystallization.

The algebraic difference between the normalized neutron-scattering intensities from the two samples can then be interpreted in terms of a compositionally weighted sum of the subset of partial pair-correlation functions in which Ca is an origin atom, as shown in investigations of the structure of ionic liquids by Soper *et al.*⁸ and Enderby and Neilson⁹. If $\Delta(Q)$ represents the normalized difference in neutron intensity scattered from the two specimens after absorption, multiple scattering and Placzek corrections, then

$$\Delta(Q) = A(S_{\text{CaCa}}(Q) - 1) + B(S_{\text{CaSi}}(Q) - 1) + C(S_{\text{CaO}}(Q) - 1) + D(S_{\text{CaAl}}(Q) - 1).$$

Here, $Q = 4\pi \sin \theta / \lambda$, with 2θ the scattering angle and λ the neutron wavelength, and the coefficients A , B , C and D are functions of the atomic fractions, c , and the coherent scattering lengths, b , of the constituent atoms. Specifically, $A =$

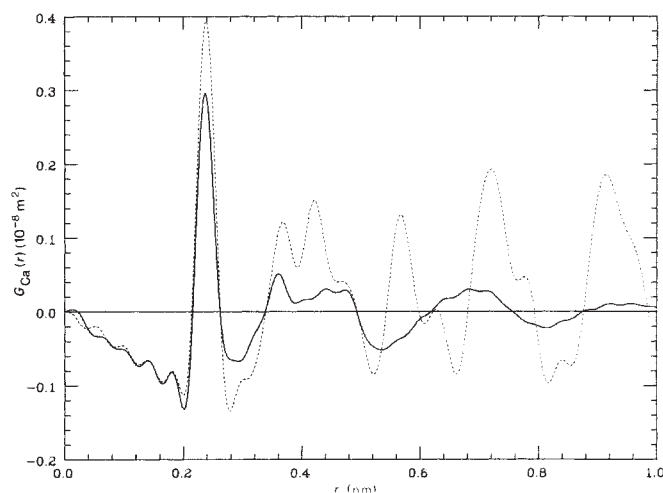


Fig. 2 Fourier transform of the corrected $\Delta(Q)$ data of Fig. 1, expressed as $G_{\text{Ca}}(r)$ (full line). The dashed line represents $G_{\text{Ca}}(r)$ for crystalline CaSiO_3 calculated with gaussian broadening parameters of $\sigma = 0.010$ nm for the first peak and $\sigma = 0.020$ nm for the remainder. The experimental data has been obtained by a Fourier transformation of $\Delta(Q)$ truncated at 148.8 nm^{-1} and a convolution of the computed curve with a Bessel function, J_0 , to reproduce the 'termination ripples' associated with a transform over limited reciprocal-space data.

$c_{\text{Ca}}^2 (b^2 - b'^2)$, $B = 2c_{\text{Ca}}c_{\text{Si}}b_{\text{Si}}\Delta b$, $C = 2c_{\text{Ca}}c_{\text{O}}b_{\text{O}}\Delta b$, $D = 2c_{\text{Ca}}c_{\text{Al}}b_{\text{Al}}\Delta b$, where $\Delta b = b - b'$, b and b' being the scattering lengths for the two Ca isotopes.

Measurements of the scattered neutron intensity at room temperature were recorded using the D4 diffractometer at the ILL Grenoble. Samples consisted of ~ 5 g of coarse powders of the crushed glasses and typical counting times were 18 h. The corrected reciprocal-space difference function, $\Delta(Q)$, (Fig. 1) has a good signal-to-noise ratio over the whole range of Q . The high- Q end of these data is, of course, similar to that obtainable by EXAFS but the region below $\sim 50 \text{ nm}^{-1}$ corresponds to the near-edge region of the X-ray absorption spectrum; the latter can be interpreted only indirectly and with some difficulty at present. But it is precisely this region that is related to correlations beyond the first Ca–O shell, so that neutron scattering is superior to EXAFS in providing this information for disordered solids.

The Fourier transform of $\Delta(Q)$ gives a weighted partial pair-correlation function $G_{\text{Ca}}(r)$, which is shown in Fig. 2 and is defined as:

$$G_{\text{Ca}}(r) = 2/\pi \int_0^\infty Q \Delta(Q) \sin(Qr) dQ \\ = AG_{\text{CaCa}}(r) + BG_{\text{CaSi}}(r) + CG_{\text{CaO}}(r) + DG_{\text{CaAl}}(r)$$

where

$$G_{ij}(r) = 4\pi r \rho_0 (g_{ij}(r) - 1)$$

and

$$g_{ij}(r) - 1 = (2\pi^2 r \rho_0)^{-1} \int_0^\infty Q (S_{ij}(Q) - 1) \sin(Qr) dQ$$

Here ρ_0 is the average atomic density. In addition to a well-defined first peak at 0.237 nm , which is almost the identical value found in crystalline CaSiO_3 (wollastonite-2M or parawollastonite¹⁰⁻¹²), a split second peak and a third peak are also seen. The distribution function shows a strong qualitative resemblance to that of *c*- CaSiO_3 , at least as far as 0.5 nm ; the first-neighbour (Ca–O) distribution function is clearly altered in detail, however, and the similarities and differences are both revealing. In *c*- CaSiO_3 , six oxygens lie between 0.224 and 0.254 nm (refs 10–12), with a mean and standard deviation of 0.240 and 0.011 nm respectively, allowing a contribution of 0.005 nm

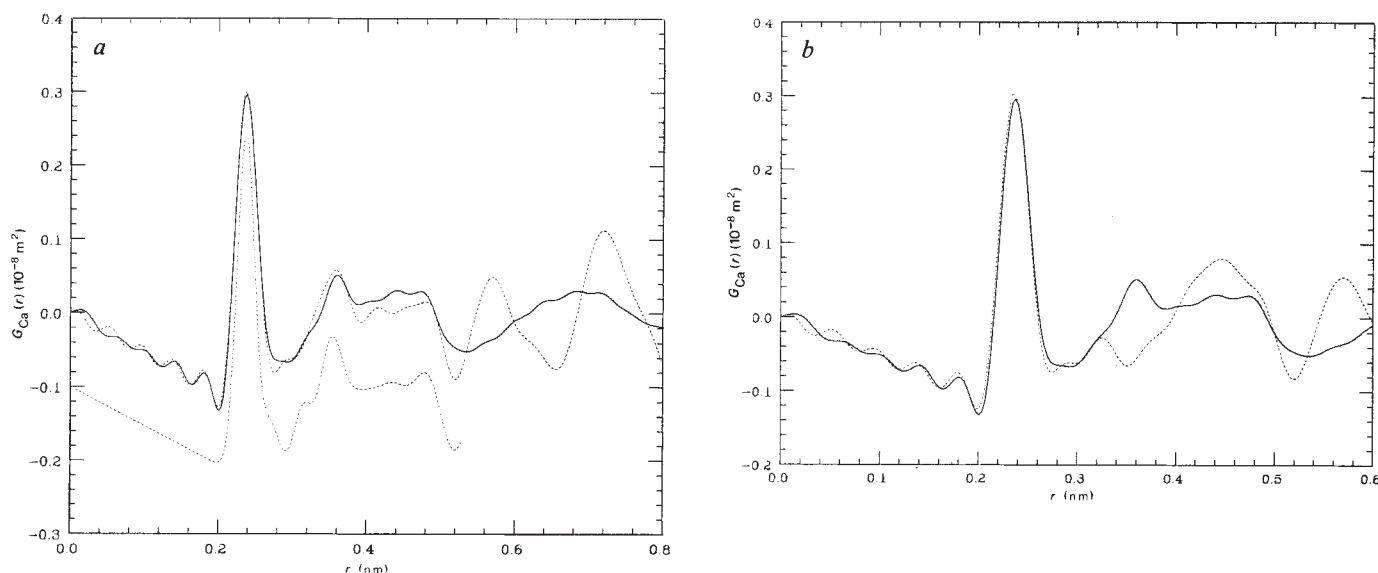


Fig. 3 Experimental data for $G_{\text{Ca}}(r)$ (full line) and a fit to the first and second peaks (dashed line) obtained by modifying the pair distribution functions for $c\text{-CaSiO}_3$. Specifically, the first peak is modelled by a gaussian function at 0.236 nm of weight 4.5 and $\sigma = 0.0115$ nm, and smaller peaks are added at 0.245, 0.265, 0.275 and 0.290 nm with weights 0.5, 0.5, 0.667 and 0.1, all with $\sigma = 0.012$ nm. The second peak has also been modelled more crudely, by broadening and shifting the Ca-O distribution by small amounts (typically <0.02 nm) and with a shift of the peak of the Ca-Ca distribution at 0.0363 nm to 0.0356 nm. The contribution from the small amount of Al has been added to that of the chemically similar Si. The lower dashed curve (displaced by 0.1) shows $G_{\text{Ca}}(r)$ before convolution by a peak-shape function commensurate with truncation of the scattering data at $Q = 148.8 \text{ nm}^{-1}$. **b**, Experimental data for $G_{\text{Ca}}(r)$ (full line) and values calculated as for **a** (dashed line) but with the Ca-Ca first peak moved from 0.35 nm to 0.44 nm, corresponding to the distance expected for random substitution of calcium ions in this glass. (For greater realism, the Ca-Ca distribution has also been broadened by a gaussian with $\sigma = 0.022$ nm, equal to that for the Ca-O(2) distribution.)

for thermal motion. One of the three distinct Ca sites has a further oxygen neighbour at 0.264 nm, giving a total coordination number of 6.33. The total Ca coordination number in the glass is 6.15 ± 0.17 , the errors being largely systematic and associated with uncertainties in Δb and composition. This value has been estimated in two ways: by a direct computation of the area corresponding to the first peak in $G_{\text{Ca}}(r)$ (out to the minimum at 0.285 nm) and by fitting the experimental distribution with a series of gaussian components, convoluted with a peak-shape function (a Bessel function, J_0) appropriate for termination of the reciprocal-space data at about 150 nm^{-1} . The resulting fit is shown in Fig. 3a together with the computed value of $G_{\text{Ca}}(r)$ before convolution. The latter gives a more realistic impression of the Ca-O bond-length distribution: the sharp central component corresponds to 5.2 atoms with a peak at 0.237 nm and a standard deviation of 0.0123 nm. The additional disorder-induced or static broadening for the glass can be obtained by subtracting (in quadrature) the contribution from the Ca-O distribution in $c\text{-CaSiO}_3$, giving a value $\sigma_s = 0.006$ nm. This indicates a well-ordered environment for almost 85% of the oxygen atoms in the first coordination shell: the static broadening is only a little larger than that associated with the Si-O first neighbour shell (0.005 nm). The remaining oxygen atom occupies a broad tail stretching from about 0.25 to 0.285 nm.

It seems reasonable, therefore, to envisage the coordination polyhedron around Ca as being mainly ordered, and probably octahedral, as in the crystal. But about one-sixth of the Ca-O bonds are significantly longer than those in the crystal, with no indication whether such long bonds are uniformly distributed or are concentrated in 'defect' regions.

Interpretation of higher-neighbour peaks is somewhat less certain. The positions of the first component of the split second peak at 0.35 nm corresponds to peaks in the Ca-Si and Ca-Ca distributions in $c\text{-CaSiO}_3$ and the second component at 0.44 nm has a counterpart in the Ca-O(2) distribution. An improved fit to the experimental data from 0.3 to 0.5 nm, shown in Fig. 3a, was produced by slightly varying the positions and breadths of the Ca-Ca and Ca-Si distributions, with more radical alterations

to Ca-O distances. The most critical test of the extent of order is afforded by the Ca-Ca partial. For random substitution, the mean Ca-Ca distance for this composition would be 0.44 nm, and consequently, direct experimental determination of this partial pair-correlation function would be especially interesting. Deconvolution of the peak at 0.35 nm, assigned to Ca-Si and (perhaps) Ca-Ca neighbours, is not possible, but the effects of (artificially) moving the Ca-Ca peak to 0.44 nm can be readily simulated. Figure 3b shows $G_{\text{Ca}}(r)$ computed as for Fig. 3a but with the first peak of the Ca-Ca distribution moved to 0.44 nm and broadened somewhat. The close correspondence between experiment and simulation is now lost. There is a strong possibility, then, that Ca atoms are coordinated by oxygen as octahedra that adopt the edge-sharing arrangement characteristic of the crystal.

It should be stressed that this work does not support a micro-crystalline model for the glass, because of the pronounced differences between the distributions for glassy and crystalline CaSiO_3 (Fig. 2) beyond about 0.5 nm.

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¹⁵N-depleted N₂O as a product of nitrification

Naohiro Yoshida*

Department of Chemistry, Tokyo Institute of Technology,
O-okayama, Meguro-ku, Tokyo 152, Japan

Recent interest in the role of nitrous oxide in the natural nitrogen cycle has focused on its production through the nitrification process¹. Nitrous oxide depleted in ¹⁵N has been found in the shallow water column of the eastern tropical Pacific Ocean², suggesting that ¹⁵N is depleted during N₂O production. Here we report significant ¹⁵N depletion in N₂O produced by bacterial nitrification, implying that N₂O is produced via NO₂⁻ rather than directly from NH₄⁺ and that N₂O should be very depleted in ¹⁵N where nitrification is the predominant production process for N₂O. The magnitude of the isotope depletion is so large that this work should lead to further use of data on the natural abundance of ¹⁵N for assessing the sources and sinks of nitrogenous compounds.

The distribution of ¹⁵N (refs 2, 3) and ¹⁸O (refs 4, 5) in atmospheric and oceanic N₂O provides useful information on its global sources and sinks. Kinetic isotope-fractionation factors in some principal routes to the production of N₂O—for example, from NH₄⁺ by bacterial nitrification—are not presently known, while those for routes to the production of NO₂⁻ and NO₃⁻ from NH₄⁺ by bacterial nitrification are available^{6,7}. Here we investigate the nitrogen-isotope behaviour of N₂O during nitrification. Because N₂O has been found to be depleted in ¹⁵N in the oceanic sub-surface water, in which N₂O is considered to be produced by nitrification², it may be inferred that ¹⁵N-depletion is a characteristic of the nitrification process in general.

Experiments were carried out using a pure culture of *Nitrosomonas europaea* (strain No. ATCC 19718). This was grown with forced aeration in the culture medium, whose composition is described elsewhere⁸. It initially contained 38 mmol l⁻¹ NH₄⁺, which was used as the nitrogen-isotope standard throughout this study. After two weeks of growth, cells were harvested by centrifuging (7,500 r.p.m.) at 5°C. Two litres of the culture medium yielded ~0.2 g of wet cells. Cells were washed with fresh medium, to remove nitrogen-isotope-fractionated NH₄⁺ and NO₂⁻, and inoculated into the culture medium. The cell density at the start of incubation was ~1.6 × 10⁷ cells per ml, determined by counting the cells dyed with acridine orange on a fluorescent microscope. About 600 ml of the medium was transferred to an incubation flask (~1,140 ml), which was attached to an incubation system, details of which are described elsewhere⁹. The incubation system was thoroughly evacuated and pure oxygen was then introduced. Incubation experiments were carried out under oxygen pressures of 0.01, 0.03 and 0.19 atm. The oxygen pressure was automatically maintained

constant to within ±0.5 mm Hg by supplying fresh oxygen from the electrolysis of CuSO₄ solution. The flask was incubated under dark and stirring conditions at 28 ± 0.5 °C.

A small fraction (~70 ml) of the gases in the incubation system (total volume ~2,020 ml) was periodically sampled by isolating sample tubes. The culture medium was sampled at the end of incubation. N₂O and NO in the gas phase were separated from each other by means of their vapour pressure difference at liquid-air temperature, and were reduced respectively to N₂ by passing over heated copper (ref. 3). NH₄⁺ and NO₂⁻ in the liquid phase were converted to N₂ by steam distillation, reduction with Devarda's alloy and subsequent oxidation with KBr (ref. 10). The N₂ thus converted from each nitrogen compound was purified over heated Cu and Cu₂O, recovered into a small volume to determine its amount manometrically, and measured for the nitrogen-isotope ratio on a VG 602C ratio mass spectrometer (VG-Isotopes Ltd, Winsford, UK). The nitrogen-isotope ratio is expressed as the ‰ difference (δ¹⁵N) from the standard pre-incubation NH₄⁺, for which δ¹⁵N with respect to atmospheric N₂ was -13.4 ± 0.1‰.

In all the experiments, the N₂O produced was depleted in ¹⁵N by lower than -60‰ (Figs 1a-c). This extreme degree of ¹⁵N-depletion has never before been observed for the microbial alteration of nitrogenous compounds¹¹. Table 1 shows the average production rate for three nitrogen species. The rate for N₂O is rather constant, while those for NO₂⁻ and NO decrease at lower oxygen pressure. The results agree well, in general, with those from other studies, although the fraction of N₂O among the nitrogen species produced at oxygen pressures of 0.03 and 0.19 atm is slightly higher than previously reported values^{12,13}. The α(N₂O) and α(NO₂⁻) values in Table 1 were calculated using the Rayleigh equation, on the assumption that N₂O and NO₂⁻, respectively, is the single product. The α(NO₂⁻) in this study is slightly closer to unity than those in the literature^{6,7}, probably owing to a higher yield of N₂O in the present study; that is, a higher yield of ¹⁵N-depleted N₂O results in greater ¹⁵N-enrichment of NO₂⁻ and thus an α(NO₂⁻) value closer to unity. Correspondingly, α(NO₂⁻) is closest to unity at lower oxygen pressures, where more N₂O is produced.

Among the proposed reaction schemes for nitrification, the one proposed by Ritchie and Nicholas⁸ is the most generally accepted. However, whether N₂O is produced directly from NH₄⁺ or by intermediate production of NO₂⁻ is yet to be resolved. For example, using ¹⁵N-tracer kinetics, Poth and Focht¹⁴ argued for N₂O production by reduction of NO₂⁻, which is produced from NH₄⁺ by *N. europaea*. On the other hand, Hynes and Knowles¹⁵ reported evidence for the direct oxidation of NH₄⁺ to N₂O by *N. europaea* in mixed-cell experiments with *Nitrobacter winogradskyi*.

If N₂O is produced from NO₂⁻, the overall reaction is similar to two-step steady-state reactions¹⁶. In this case, α(N₂O) would be expressed as the product of two kinetic nitrogen-isotope fractionation factors, namely the α(NO₂⁻) in this study and α'(N₂O), the kinetic isotope-fractionation factor for reduction of NO₂⁻ to N₂O. The α'(N₂O) value in Table 1 was calculated

Table 1 Kinetic data on the nitrification by *N. europaea*

P(O ₂) (atm)	N ₂ O	Production rate*		N ₂ O/N (%)†	NH ₄ ⁺ reacted (%)§	Calculated fractionation factor‡		
		NO (µg of N per h)	NO ₂ ⁻			α(N ₂ O)	α(NO ₂ ⁻)	α'(N ₂ O)
0.01	11	5.2	89	11	3.5	1.0604	1.0246	1.0349
0.03	11	3.1	82	11	6.4	1.0672	1.0299	1.0362
0.19	7.9	82	150	3.3	96	1.0684	1.0320	1.0353

* For N₂O and NO, rates are calculated from Fig. 1 as the average through the incubation period; for NO₂⁻, the rate is given by the amount found in the medium at the end of the incubation divided by the incubation time.

† Fraction of N₂O in the total of all nitrogen species produced.

‡ α is expressed as the ratio of rate constants for isotopic species, that is, k(¹⁴N)/k(¹⁵N); α(N₂O) and α(NO₂⁻) were obtained from Fig. 1.

§ Fraction of NH₄⁺ reacted at the end of the incubation.

|| See text for definition of α'(N₂O).

* Present address: Department of Earth Sciences, Toyama University, Gofuku, Toyama 930, Japan.

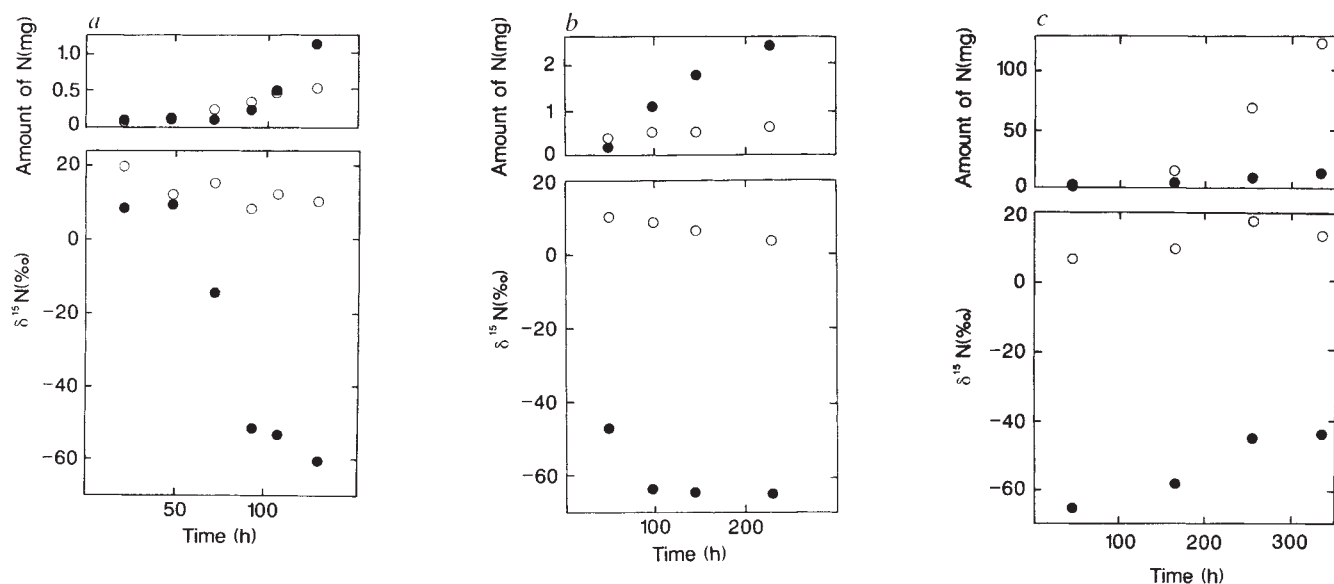


Fig. 1 Temporal change in amount (top) and $\delta^{15}\text{N}$ (bottom) of N_2O (solid circles) and of NO (open circles) produced by *N. europaea*. Oxygen pressure: a, 0.01 atm; b, 0.03 atm; c, 0.19 atm.

by dividing $\alpha(\text{N}_2\text{O})$ by $\alpha(\text{NO}_2^-)$. It is rather constant and similar to those reported for several bacterial species during denitrification^{7,11}, suggesting that N_2O is produced by the NO_2^- reduction step rather than directly by the NH_4^+ oxidation step. The large ^{15}N -depletion in N_2O observed here therefore appears to be a result of a two-step depletion process.

As shown in Figs 1b and c, N_2O is apparently in isotope-exchange equilibrium with NO over 100 h of incubation. The equilibrium isotope-fractionation factor for $\text{N}_2\text{O}/\text{NO}$ has been calculated by Richet¹⁷ to be 1.0378 at 28 °C, which indicates that N_2O is expected to be more enriched in ^{15}N than is NO . The present isotope relationship is therefore quite different from that expected from such isotope equilibrium, and must be a result of kinetic isotope-fractionation. NO reacts with oxygen to produce NO_2 , which dissolves in water to produce NO_2^- and NO_3^- . Since no data are available for kinetic nitrogen-isotope fractionation factors for oxidation of NO and dissolution of NO_2 , the isotopic behaviour of NO cannot be discussed.

NO cannot be ignored in a consideration of the nitrogen-isotope kinetics of nitrification, particularly at high oxygen pressures, where NO is the predominant gaseous component. The observed ^{15}N -depletion of N_2O is so large, however, that the direction and the extent of kinetic nitrogen-isotope fractionation during N_2O production by nitrification would not change even if NO is taken into consideration.

Wherever N_2O is produced actively by nitrification, ambient N_2O should be greatly depleted in ^{15}N . The lowest $\delta^{15}\text{N}$ value of N_2O observed in the eastern tropical Pacific Ocean was, however, 3.3‰ (ref. 2) and in the western North Pacific Ocean was 3.4‰ (N.Y. *et al.*, in preparation). These values are significantly higher than those expected from the present study on the assumption that oceanic N_2O in shallow water columns is produced predominantly by nitrification, implying that the role of nitrification in the production of N_2O in the oceans has been previously overestimated.

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Climatically forced organic carbon burial in equatorial Atlantic and Pacific Oceans

Mitchell Lyle

College of Oceanography, Oregon State University, Corvallis, Oregon 97331, USA

Over the past ten years, sedimentary geochemists have noted that climate changes associated with Pleistocene glaciations have had a pronounced effect on the rate of organic carbon burial found in the tropical oceans^{1–4}. In particular, two to five times higher organic carbon accumulation rates have been observed for the 18 kyr BP glacial maximum than for the Holocene in several short sediment cores from both oceans (see Fig. 1). Here I present 300-kyr time series for organic carbon mass accumulation rate from four sediment cores, two each from the equatorial Pacific and Atlantic, and show that orbital forcing of global climate has strongly affected the rate of organic carbon burial in the equatorial oceans.

Increased burial of organic carbon (C_{org}) may be due either to increased primary productivity and increased rain of organic matter out of the euphotic zone or to low-oxygen bottom waters inducing less degradation of the organic fraction after it has reached the sea floor. The weight of evidence favours the hypothesis that increased C_{org} mass accumulation rate (MAR) in the sediments is due primarily to increases in palaeoproductivity^{4,5}. There is no significant coherence between C_{org} MAR and modelled bottom-water oxygen in the eastern Pacific, indicating that bottom-water oxygen is not important in the creation of the C_{org} MAR record⁴. In contrast, the C_{org} MAR

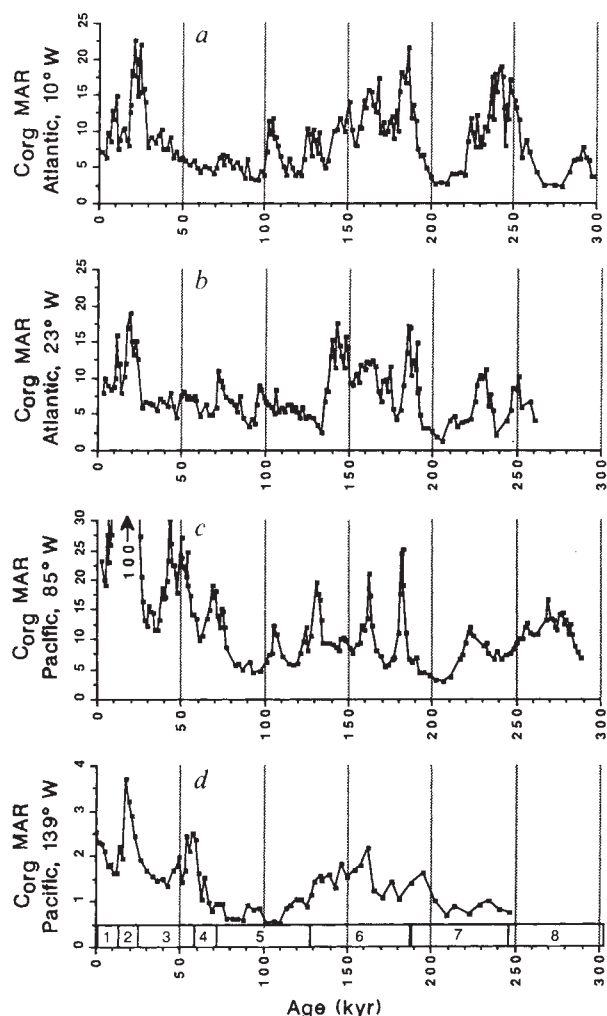


Fig. 1 Organic carbon MAR (in units of $\text{mg cm}^{-2} \text{kyr}^{-1}$) time series from *a*, W8402A-14GC ($0^{\circ}57.2' \text{N}$, $138^{\circ}57.3' \text{W}$, 4,287 m), *b*, V19-28 ($2^{\circ}28' \text{S}$, $84^{\circ}39' \text{W}$, 2,670 m), *c*, V30-40 ($0^{\circ}12' \text{S}$, $23^{\circ}09' \text{W}$, 3,620 m) and *d*, RC24-16 ($5^{\circ}02.3' \text{S}$, $10^{\circ}11.5' \text{W}$, 3,481 m). An age scale was developed for each core through the use of oxygen isotope stratigraphy^{8,9}. Isotope stage boundaries are shown on *a*. MARs were calculated using the methods described in Lyle *et al.*⁴ for the Pacific and using a bulk density estimate described in Curry and Lohmann⁷ in the Atlantic. Each core has a maximum episode of organic carbon MAR associated with the last glacial maximum, at 18 kyr. In addition, the Atlantic cores have pronounced maxima between 130 and 200 kyr.

record is correlated with the depositional fluxes of biogenic opal and calcite, indicating that all three are linked by primary productivity in the euphotic zone⁴. Increased abundance and size of benthic forams are coincident with the most recent C_{org} MAR maximum, pointing to an increased benthic food supply⁵. Finally, the records of redox-sensitive elements are not in accord with the low-oxygen bottom-water hypothesis⁵. C_{org} MAR and productivity are probably not correlated linearly, however, because preservation of organic matter depends upon the sedimentary redox environment, which in turn depends upon the flux of organic matter to the sea floor.

Few long, high-resolution records of C_{org} MAR exist in either ocean. In the equatorial Pacific, between 5°N and 5°S , Rea *et al.*⁶ have presented a 420-kyr low-resolution record (~ 10 -kyr data spacing) and Lyle *et al.*⁴ present two such records extending ~ 250 kyr with 1–3-kyr sample spacing. In the tropical Atlantic, Müller *et al.*² have estimated palaeoproductivity in several cores from the eastern basin, with the southernmost core at about 6°N on the Sierra Leone Rise (Core 13519-2). The estimated

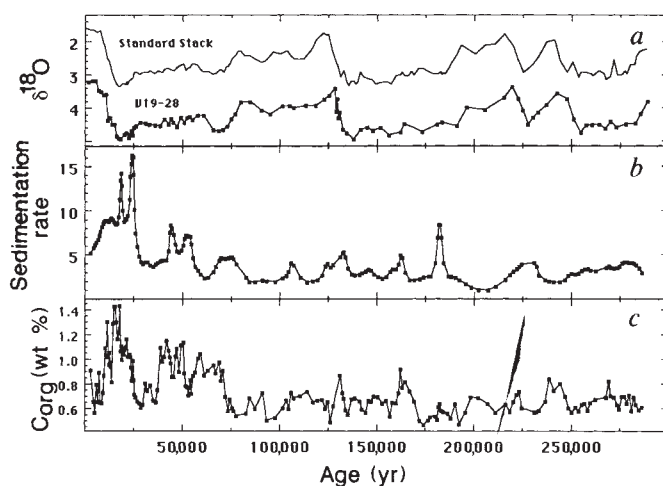


Fig. 2 Example of how C_{org} MAR time series are produced, using the core V19-28. A $\delta^{18}\text{O}$ record from V19-28 is correlated with a standard $\delta^{18}\text{O}$ stack⁹ (*a*) and the resulting age–depth relationship is differentiated to obtain the sedimentation-rate time series (in cm kyr^{-1}) (*b*). This sedimentation-rate information is multiplied by the raw C_{org} weight percentage (*c*) to obtain the C_{org} MAR shown in Fig. 1.

palaeoproductivity record from the latter core, although of low resolution, indicates that productivity was higher in glacial intervals than in interglacials. Curry and Lohmann⁷ have presented average C_{org} MAR determinations for a few representative time intervals during the past 160 kyr from cores on the Sierra Leone Rise, primarily north of 4°N . Their sparse data are similar to that of Müller *et al.*², except in the Holocene.

The C_{org} MAR records, shown in Fig. 1, are from two equatorial Atlantic and two equatorial Pacific cores, all from beneath the South Equatorial Current in each ocean. The C_{org} MAR records were created as follows. First, oxygen isotope records from each core were correlated in detail to standard oxygen isotope age scales developed by the SPECMAP project^{8,9} (Fig. 2*a*). Cross-spectral analysis was performed on each core's resultant $\delta^{18}\text{O}$ time series and the standard $\delta^{18}\text{O}$ stack in order to ensure that the two isotope time series are in phase and that spurious frequency information has not appeared from a bad correlation. The age/depth curves for each core were then differentiated to obtain sedimentation rates (Fig. 2*b*). The sedimentation rates were multiplied by the dry bulk density and by the C_{org} content (Fig. 2*c*) to obtain C_{org} MAR⁴.

Sedimentation rates for the cores are sensitive to errors in correlation to the standard $\delta^{18}\text{O}$ stack and in the absolute reliability of the standard stack ($\sim \pm 2.5$ kyr (ref. 9)). Errors in the timescale of the standard stack will not tend to introduce serious sedimentation rate errors but may introduce high-frequency noise in the time series. This results from the fact that the error in determining the age difference between points on the timescale is the same no matter how widely spaced they are; the two SPECMAP $\delta^{18}\text{O}$ timescales^{8,9}, developed from independent sets of sediment cores, provide examples of this property. Isotope stage 5, 56 kyr long, is divided into six substages, each of ~ 10 kyr. The length of each substage varies between the two timescales by up to 4 kyr and can cause sedimentation rate differences of as much as 60%, depending on the timescale. But all the differences are compensated for within the next substage, so that the timespan of stage 5 (and average sedimentation rate difference) varies by only 2%. Serious sedimentation rate errors may result from poor correlation of the individual core's $\delta^{18}\text{O}$ record to the stack, so that this aspect must be approached with care.

In general, each C_{org} MAR record has maxima during the glacial episode marked by oxygen isotope stage 6 and minima

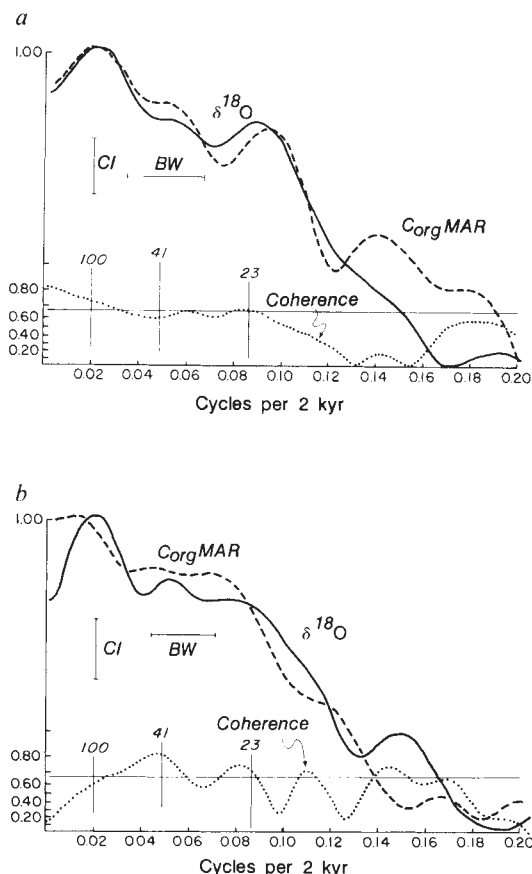


Fig. 3 Cross-spectral analysis of C_{org} MAR and oxygen isotope records for the Atlantic core V30-40 (a) and the Pacific core V19-28 (b). The dashed line represents the C_{org} MAR time series and the solid line is that of $\delta^{18}O$. The dotted line gives the coherence between the two time series. The two are coherent at >80% confidence interval (CI) when above the horizontal dividing line. Also marked are the Milankovitch orbital periods: eccentricity (100 kyr), tilt (41 kyr) and precession (23 kyr). Both the Atlantic and Pacific C_{org} MAR time series have coherence with $\delta^{18}O$ near or greater than the 80% CI at the Milankovitch frequencies. BW denotes the bandwidth.

during the interglacial stage 5. Each record has its strongest maximum at 18 kyr, during the peak of the last glacial episode. In the Pacific ocean, the 18-kyr maxima are 1.5 to 3 times larger than any other peak in the record, but in the Atlantic they are of about the same size as events in isotope stage 6. Elevated C_{org} burial is not strictly associated with the major glacial episodes, nor do C_{org} -depleted intervals occur only in interglacials. For example, elevated C_{org} deposition is associated with a short-lived oxygen isotope excursion at about 228 kyr in

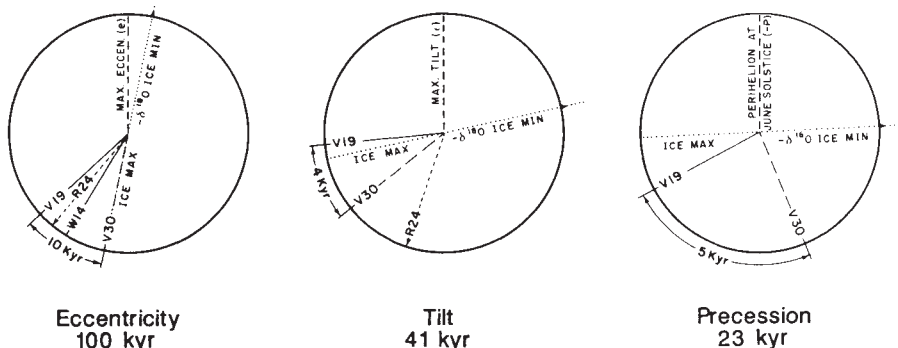
three of the four cores. With the exception of stage 2, the major glacial $\delta^{18}O$ episode during stages 2, 3 and 4 in the Atlantic cores has low C_{org} MAR, whereas the Pacific cores have high C_{org} MAR throughout this interval. Clearly, regional differences in productivity or carbon degradation have left strong imprints on the records. Such differences will be useful eventually for testing the response of palaeoclimatic models by their specific regional predictions for upwelling and advection.

A common pattern of C_{org} MAR can be discerned despite the superimposed regional pattern, as can be shown by cross-spectral analysis of V30-40, from 23° W in the Atlantic, and V19-28, from 85° W in the Pacific. Each C_{org} MAR record is compared to the core's $\delta^{18}O$ record, which is a measure of global ice volume (Fig. 3). Because each record is short relative to the Milankovitch orbital variations, I chose a wide bandwidth for the spectral analysis to increase the confidence in the results. The V30-40 C_{org} MAR record contains power at all the major orbital frequencies in about the same proportion as the $\delta^{18}O$ record. The $\delta^{18}O$ and C_{org} MAR time series are coherent in all the Milankovitch frequencies at or near the 80% confidence interval, with the poorest coherence at 41 kyr (<80% confidence interval). The records are in phase at the 100-kyr eccentricity period, but C_{org} MAR leads at the 41-kyr tilt and 23-kyr precession periods by 24° and 100° respectively. The V19-28 C_{org} MAR record contains a single very large burial event at 18 kyr that imposes power in the low-frequency end of the spectrum. Nevertheless, the C_{org} MAR and $\delta^{18}O$ records are coherent at the tilt and precession frequencies. Removal of the 18-kyr C_{org} MAR spike greatly improves the coherence at the 100-kyr period but weakens the coherence at the other two. The $\delta^{18}O$ record leads that of C_{org} MAR in V19-28 by 64° at 100 kyr and by 54° at 41 kyr, but lags behind C_{org} MAR by 17° at 23 kyr.

Cross-spectral analysis between a standardized and stacked signal of the orbital climatic forcing over the last 300 kyr (ETP; see below and ref. 8) and the C_{org} MAR time series provides another method to examine the phase relationships of the C_{org} MAR time series between oceans and to examine ties with Pleistocene climate. ETP is a stack of standardized sets of the eccentricity, tilt and precession parameters aligned so that maxima in each correspond to maximum Northern Hemisphere summer insolation. The data are presented as phase wheels, one each for eccentricity, tilt and precession (Fig. 4) with maximum Northern Hemisphere summer insolation at the vertical (12 o'clock) position. Clockwise displacements move forward in time; if a record has a 90° phase lag with respect to the eccentricity maximum, it appears at the 3 o'clock position. On each phase wheel I present data from those cores with a coherence >0.5 for the respective orbital parameters. For comparison, each phase wheel also shows the phase shift between the $\delta^{18}O$ derived ice maximum and the orbital forcing components. The error in each phase angle is $\sim \pm 40^\circ$ for all the cores at 100 kyr and 41 kyr, and at 23 kyr it is $\pm 60^\circ$ for V19-28 and $\pm 30^\circ$ for V30-40.

At the 100-kyr eccentricity period, the four cores have C_{org} MAR maxima nearly in phase with ice-volume maxima. The

Fig. 4 Phase wheels of C_{org} MAR compared to orbital forcing (ETP⁸) and the $\delta^{18}O$ ice volume records, with the 12 o'clock position marking maximum Northern Hemisphere summer insolation. The dotted lines mark the phase angle of maximum ice volume with respect to the orbital parameters and solid lines are drawn to the phase angle of the C_{org} MAR maximum if coherence is >0.5 (they are not shown otherwise). V19 and W14 are the two Pacific cores V19-28 and W8402A-14GC, whereas R24 and V30 are the two Atlantic cores RC24-16 and V30-40.



phase shift between V30–40 and V19–28 is equivalent to a lag by V19–28 of 10 kyr. At the 41-kyr tilt period only three cores have coherence >0.5 , and for two of these the C_{org} MAR maxima are nearly in phase with maximum ice volume, but the Atlantic cores tend to lead the ice maximum. The phase shift between the two extreme cores is equivalent to an 8-kyr lag of C_{org} MAR in V19–28 with respect to RC 24–16. At the 23-kyr precession period C_{org} MAR maxima from the two cores with >0.5 coherence (V19–28 and V30–40) lead ice volume. V19–28 is nearly in phase with the ice volume signal, and the Atlantic C_{org} MAR record from V30–40 again leads (by 5 kyr).

For each orbital period the equatorial Atlantic Ocean C_{org} MAR in general responds ~ 5 – 10 kyr before the equatorial Pacific Ocean. Such a response pattern, if upheld by other sediment data, indicates that C_{org} burial in both oceans is forced by climate variations in some manner but not by the same specific events. C_{org} MAR variations in the Atlantic and the Pacific could be tightly linked or could be caused by two different phenomena (for example, wind-driven upwelling in one ocean and bottom-water formation in the other) as long as both are climatically sensitive and occur in a specific sequence.

Although C_{org} MAR maxima in the equatorial Atlantic and Pacific tend to be associated with ice-volume maxima, the phase relationships between the two types of records at the different orbital periods show that changes in ice volume do not directly cause changes in equatorial C_{org} MAR, as might occur if global ocean nutrient content and productivity were controlled by deposition or erosion of continental shelf sediments¹⁰. Instead, C_{org} MAR tends to lead the 23-kyr precession-induced changes in ice volume, and to lag behind the 100-kyr eccentricity-related changes. As C_{org} MAR is most probably an index of primary productivity and because all the C_{org} MAR records contain most of their variance at 100 kyr, changes in primary productivity in the equatorial oceans probably result from, rather than cause, climate changes; the latter would be viable if changes in equatorial productivity strongly affected atmospheric CO_2 .

I can thus discern patterns of C_{org} deposition common to both the equatorial Atlantic and Pacific Oceans, in sediment cores that span nearly halfway around the globe. There is also sufficient regional variation for C_{org} MAR time series to be useful in more detailed climatic studies. Elevated C_{org} MAR is associated with glacial climatic conditions, but is not directly related to ice volume. At all Milankovitch orbital periods the Atlantic reaches maxima in C_{org} MAR about 5 kyr before the maxima in the Pacific. Such behaviour implies that variations of C_{org} MAR in the equatorial Atlantic and Pacific are linked, although it is still uncertain whether the variations have a common cause or whether distinct processes in each ocean are linked by common forcing. Because they are probably an index of productivity, C_{org} mass accumulation rates will provide a means to monitor the flow of nutrients into the upper oceans and to monitor the changing pattern of productivity associated with changing climate.

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Loihi Seamount, Hawaii: a mid-plate volcano with a distinctive hydrothermal system

D. M. Karl*†, G. M. McMurtry*†, A. Malahoff*† & M. O. Garcia*

* Hawaii Institute of Geophysics, University of Hawaii, Honolulu, Hawaii 96822, USA

† Department of Oceanography, University of Hawaii, Honolulu, Hawaii 96822, USA

The initial discovery of deep-sea hydrothermal vents at the Galapagos Rift^{1,2} has resulted in a decade of exploration and experimentation. Subsequent discoveries in the Pacific^{3–8} and Atlantic⁹ ocean basins have established the importance of these features as sources of mantle-derived gases and solutes^{10–12}, and as loci for heat dissipation^{13,14}, polymetallic sulphide mineral deposition^{15,16} and geochemical exchange^{17–19}. The presence of dense communities of bacteria and specialized macrofauna^{20–22} has called into question the absolute role of sunlight as the principal energy source for marine organisms. Until recently, these deep-sea vents were known only at, or near, plate boundaries, but recent dredging^{23,24}, near-bottom-water sampling^{25–27} and sea-floor camera survey²⁸ programmes have indicated that hydrothermal vents are present at the summit of Loihi Seamount, a mid-plate, hotspot submarine volcano at the southern end of the Hawaiian island chain²⁹. Here we report results obtained from sampling two active vent fields. The fluid chemistry (especially high CO_2 and Fe), presence of alkalic lavas and iron-depositing bacteria, and absence of macrofauna suggest that mid-plate vents may differ from the hydrothermal vents found at mid-ocean ridges.

Two hydrothermal fields were found in 1987 on the upper portion of Loihi's south rift zone. Both fields are located on the eastern flanks of prominent pillow lava cones (Figs 1 and 2). Lavas from the venting areas are brecciated and strongly vesicular (20–35 volume per cent). The lavas from the northern vent field (named Pele's Vents, after the Hawaiian volcano goddess; 250 m² in area) are weakly phryic (1–2 volume per cent plagioclase, olivine and augite), alkalic in composition and de-gas vigorously when brought to the surface. The lavas from the southern vent field (named Kapo's Vents after Pele's older sister; 25 m² in area) are strongly olivine-phryic (10–15 volume per cent) and are tholeiitic in composition. Individual vents are a few to twenty centimetres across with discharge velocities of 1–10 cm s⁻¹. Earlier studies of palagonite rinds on dredged pillow lavas suggested that Loihi's alkalic lavas are older than its tholeiitic lavas²⁹, but our new results indicate that alkalic volcanism is continuing on Loihi Volcano.

The lavas in the two vent fields are mantled with red-orange 'nontronite' deposits, an assemblage of varying amounts of iron oxides, silica and iron-rich smectite^{23,24}. Thick, orange-stained bacterial mats with iron oxide coatings (Fig. 3) cover lavas on the periphery of the vent field. On lava surfaces adjacent to active vents, white deposits of elemental sulphur are present, and just beyond these are found bright orangish-red lepidocrocite deposits. The presence of elemental sulphur in the immediate vicinity of the vents suggests that oxidation of vent-water H_2S may be occurring, perhaps within the upper portion of the seamount edifice, but no H_2S has yet been detected by the water sampling programme^{30,31}.

Hydrothermal fluids were collected from Pele's Vents in February and August 1987 using piston-driven titanium samplers¹⁸. Bacterial mats were sampled using standard ALVIN tube corers. Individual vent-water samples and Niskin-bottle-collected control deep-water samples were processed immediately upon their arrival on the respective support vessels, which in extreme cases was 4–6 h from the time of *in situ* sample

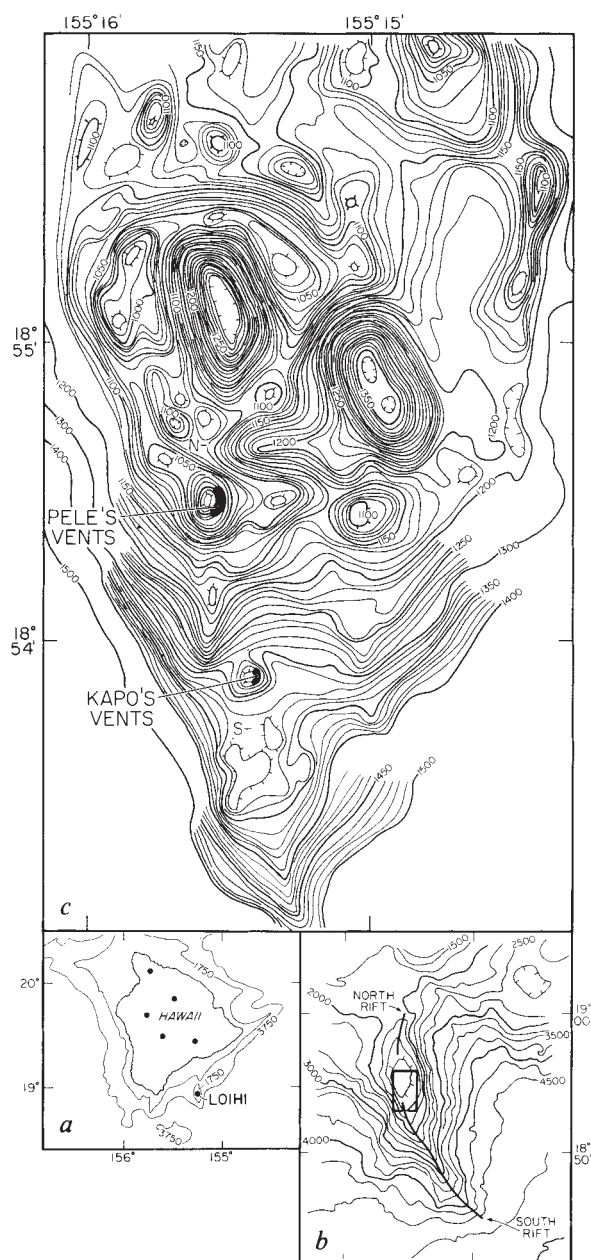


Fig. 1 Location maps for hydrothermal vents on Loihi Seamount. *a*, Location of Loihi Seamount relative to the island of Hawaii and its five shield volcanoes (the summit of each is shown by a solid circle). Bathymetric contours are in metres. *b*, Sea Beam map of Loihi (derived from a 1987 NOAA Sea Beam survey) showing the two principal rift zones and location of Fig. 1*c* (box). *c*, Detailed Sea Beam bathymetric map of the central and southern portions of the summit and upper south rift zone of Loihi. The locations of the hydrothermal vent fields are shown in black. The contour interval is 10 m. The end points for the cross-section in Fig. 2 are shown by the lines next to the N and S.

collection. All samples were subsequently analysed at our shore-based laboratories.

The chemistry of the Loihi vent waters has both similarities to and differences from other low-temperature vent fields, such as those found on the Galapagos Rift and on Axial Seamount, a hotspot volcano located on the Juan de Fuca Ridge (Table 1). Dissolved SiO_2 positively correlates with vent temperature. Other vent-water components also display linear trends with SiO_2 , suggesting a common end-member for the numerous individual vents from Pele's vent field. Relative to deep sea

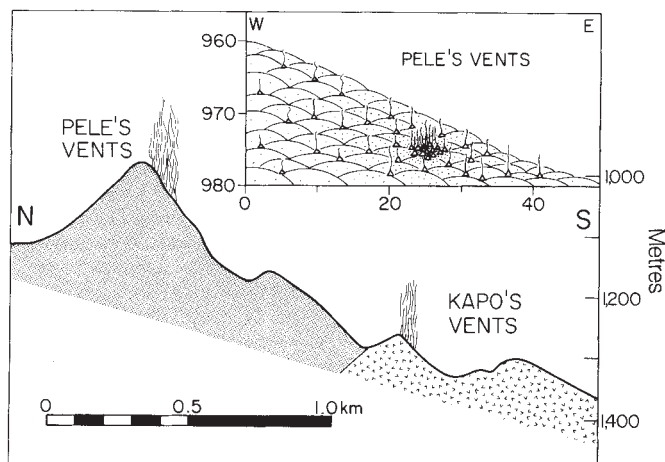


Fig. 2 Cross-section of the upper south rift zone of Loihi Seamount. Hydrothermal vents are located on the east flanks of two pillow cones. Note the large size of the (summit) cone on which Pele's Vents are located. It is larger than any other rift zone cone and is the second largest constructional feature on Loihi Seamount. The distribution of rock types is shown by the patterns: stippled—alkalic; v's—tholeiitic. Analyses (wt %) of pillow rim glasses from (1) Pele's Vents— SiO_2 : 48.8, TiO_2 : 3.7, Al_2O_3 : 13.8, FeO : 13.0, MnO : 0.2, MgO : 5.1, CaO : 10.2, Na_2O : 3.3, K_2O : 0.90, P_2O_5 : 0.43; (2) KAPO'S VENTS— SiO_2 : 49.2, TiO_2 : 2.6, Al_2O_3 : 13.4, FeO : 11.3, MnO : 0.2, MgO : 7.2, CaO : 12.0, Na_2O : 2.5, K_2O : 0.5, P_2O_5 : 0.30. *Insert* Cartoon showing the geology of Pele's Vent. Triangles show vent locations; dots show nontronite distribution; arched lines represent pillow lavas.

water, the vent waters are enriched in total CO_2 , CH_4 , NH_4 , PO_4 , Fe, Mn, Li, K, Rb, Ca, Sr and Ba and depleted in Mg, SO_4 , $[\text{NO}_3 + \text{NO}_2]$ and Cl.

The greatest difference in the composition of the Loihi vent waters relative to other low-temperature submarine vent fluids is the very high concentration of dissolved CO_2 , which is also reflected in the relatively low pH of the vent and plume waters (Table 1)^{26,27,31}. The low pH of the Loihi vent waters probably accounts for the very high dissolved iron concentrations which appear to be unique among low-temperature hydrothermal systems. The acidic vent waters and diffuse-flow regime are primary factors in creating the abundance of low-temperature, iron-rich oxide and silicate hydrothermal deposits found on the summit and rift zones of Loihi Seamount, and of the extensive bacterial mats.

Warm water samples (15–30 °C) collected at Pele's Vents contained elevated concentrations of particulate ATP (up to 118 ng l^{-1}) relative to ambient bottom seawater ($<5 \text{ ng l}^{-1}$). These values are comparable to the enrichments measured at other deep-sea hydrothermal vent sites²¹, and indicate the presence of a localized source of microorganisms. Bacteria present in water samples collected from the warm vent waters actively oxidized $^{14}\text{CH}_4$ to $^{14}\text{CO}_2$ and incorporated ^3H -adenine and ^3H -thymidine into nucleic acids when incubated at 1 atm and 30 °C, indicating active metabolism and cell growth. During the August 1987 expedition, we also discovered the presence of an active thermophilic bacterial population with high rates of metabolic activity at 1 atm and 60 °C. Based on our initial observations and collections, there appear to be at least four distinct bacterial habitats at Loihi Seamount: (1) the anoxic sub-surface vent system, (2) the region immediately surrounding the vent loci where the anoxic hydrothermal fluids are mixed with oxygenated deep-sea waters, (3) the region of extensive bacterial mat formation which is restricted to the periphery of the main hydrothermal vent field, and (4) the hydrothermal plume.

The two active vent fields and several inactive hydrothermal areas on Loihi Seamount are all devoid of the luxuriant

Fig. 3 Structure and composition of bacterial mat sampled from Loihi Seamount. *a* and *b*, Scanning electron microscopy images of individual filaments from the bacterial mat. The primary morphology is that of long (50–150 μm), thin ($\sim 1\text{--}2\ \mu\text{m}$ in diameter) filaments, without branching. Numerous non-filamentous bacterial cells were found in association with the bacterial mats. Scale marker = 5 μm . *c*, Longitudinal and cross-sectional transmission electron microscopic images of thin sections of individual bacterial filaments. The filaments are tubular with relatively constant wall diameters of 0.2–0.3 μm . Recognizable cellular materials were observed within selected filaments (see arrow). Scale marker indicates 1 μm . *d*, Energy dispersive X-ray fluorescence analysis indicates that the mat is comprised predominantly of Fe with significant contributions of Ca and P. These results were confirmed by bulk chemical measurements and by electron microprobe analyses which revealed a Fe:P ratio of 6–7:1 (by weight). Additional studies indicated that >95% of the phosphorus was hydrogenous, and that the bacterial mats were amorphous³⁴.

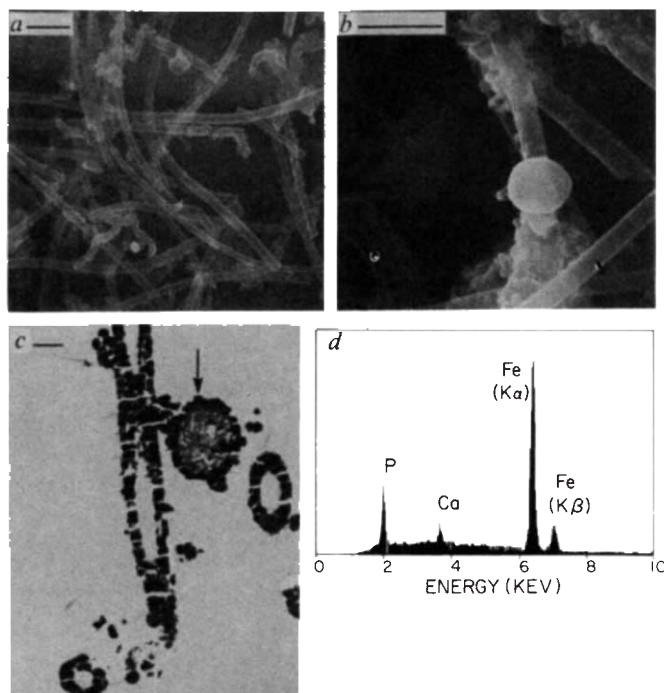


Table 1 Summary of Loihi hydrothermal vent fluid chemistry and comparison with other known deep-sea vent habitats.

Component*	Pele's Vents (30 °C)	Ambient† seawater	Vent water enrichment (+) or depletion (–) (%)	Axial Seamount‡ Vent A (29 °C)	Galapagos Rift§ composite (30 °C)
Li (μM)	36.5	23.6	+55	58.0	79.7–116.6
Na (mM)	464	455	+2	—	—
K (mM)	12.3	10.2	+21	—	10.7
Rb (μM)	2.85	1.25	+128	4.4	1.28–2.89
Mg (mM)	50.3	52.2	–4	49.39	48.4
Ca (mM)	11.2	10.3	+9	11.06	11.5–12.7
Sr (μM)	88.5	86.9	+2	92	86.6–87.3
Ba (μM)	0.71	0.07	+914	1.31	1.52–3.53
Si (μM)	1,285	123	+945	1,100	1,884
Cl (mM)	524	534	–2	531.9	524–545
SO ₄ (mM)	27.0	28.8	–6	25.94	26.2
Fe (μM)	1,010	0.05	+2 $\times 10^6$	2.6	24¶
Mn (μM)	21.1	0.01	+2 $\times 10^5$	27.7	36.2–115
CH ₄ (nM)	7,254#	7.2	+1 $\times 10^5$	—	6,910–24,200**
NH ₄ (μM)	5.18	0.72	+619	—	—
PO ₄ (μM)	3.46	2.93	+18	—	—
[NO ₃ + NO ₂] (μM)	11.9	42.1	–72	—	0.0**
ΣCO ₂ (mM)	300††	2.38	+1.3 $\times 10^4$	40	2.60–3.35‡‡
pH	5.3–5.5§§	7.5	+1.6 $\times 10^4$	6.18	6.65

* Water samples for major and minor inorganic constituents were processed on board ship by filtration through prewashed 0.2 μm Nuclepore filters followed by pH adjustment to ≤ 2.0 with ultrapure nitric acid. Na, K, Rb, Ca, Mg, Si, and for concentrated vent waters, Fe and Mn were determined by flame atomic absorption spectrophotometry. Minimum precisions based on multiple determinations of both samples and standards are: Na (1%); K (4%); Rb (10%); Ca (2%); Mg (2%); Si (3%); Fe (2%); Mn (2%). Lithium (4%) was determined by flame atomic emission spectrophotometry. Ba, Fe and Mn in ambient waters were determined by graphite furnace atomic absorption spectrophotometry, following preconcentration for Fe and Mn. Minimum precisions are: Ba (30%), Fe (10%), Mn (10%). SO₄ (1.5%) was determined by ion chromatography. Chloride (0.1%) was determined by potentiometric titration with AgNO₃. For dissolved nutrient determinations, water samples were filtered through combusted glass fibre filters and stored frozen for subsequent analysis by standard autoanalyser procedures.

† Sample collected with a 5-litre Niskin bottle at 980 m near Pele's Vents; water temperature = 3.5 °C.

‡ Data from Chase *et al.* 1985 (ref. 6).

§ Extrapolated values to 30 °C from T versus Si data of Edmond *et al.*^{32,33}.

|| Sr analyses by isotope dilution, mass spectrometry courtesy of J. D. Macdougall.

¶ Highest value at 9 °C for Clambake Field; all other Galapagos Rift fields display mid-range Fe maxima and decrease at higher temperatures. Reported as total dissolved metal. From Edmond *et al.*³³.

Extrapolated value to 1,285 μM Si from CH₄ versus Si data of Karl *et al.*³⁴.

** Extrapolated values to 30 °C from data of Lilley *et al.*¹¹.

†† Extrapolated value to 1,285 μM Si from ΣCO₂ versus Si data of Karl *et al.*³⁴.

‡‡ Extrapolated values to 30 °C from data of Edmond *et al.*³⁵.

§§ From Edmond *et al.*³¹.

||| From Gamo *et al.*²⁷.

macrobenthic communities that have become the hallmark of analogous mid-oceanic ridge sites studied to date²². This is of particular interest because the bacterial populations, which are presumed to form the base of the hydrothermal vent food-webs²⁰, are present in high concentrations at Pele's Vents. Whether this apparently anomalous condition is the result of isolation and the relatively young age of Loihi compared with mid-ocean ridge systems, of the failure of organisms to recruit on sediment-covered substrata and to withstand the unusual physical and chemical conditions of the Loihi vent fields, or of acute toxicity caused by the elevated carbon dioxide or dissolved metal concentrations, is unknown.

The extremely high concentration of dissolved carbon dioxide and iron in the Loihi vent waters, the presence of iron-oxidizing bacterial populations and the absence of benthic macrofauna suggest that mid-plate, hotspot hydrothermal systems may be fundamentally different from those on mid-ocean ridges. Hydrothermal vents have not been previously reported from alkalic volcanoes, but other mid-plate alkalic seamounts (for example, Macdonald Seamount) probably have active hydrothermal vents. Furthermore, our discovery of active vents in a mid-oceanic plate setting requires that we revise upward the global flux of hydrothermal fluid emissions and their total contribution to element cycling and associated deep-sea bacterial productivity. One advantage of the location of the Loihi Seamount vents (<40 km from land) is the potential for establishing a permanent laboratory for seafloor observations and *in situ* experimentation.

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Late Archaean terrane accretion in the Godthåb region, southern West Greenland

C. R. L. Friend*, A. P. Nutman† & V. R. McGregor‡

* Department of Geology, Oxford Polytechnic, Oxford OX3 0BP, UK

† Department of Earth Sciences, Memorial University of Newfoundland, St John's, Newfoundland, A1B 3X5, Canada

‡ Atammik, 3912 Sukkertoppen, Greenland, Denmark

Tectono-stratigraphic terranes—fault-bounded blocks of the Earth's crust characterized by a geological history distinct from that of adjacent terranes¹—are now widely recognized in Phanerozoic and Proterozoic orogenic belts, and their present configurations are thought to result from plate-tectonic processes similar to those in operation today²⁻⁴. There has been much debate about whether such processes operated in Archaean times^{5,6}. Here we present new data from the Archaean high-grade gneiss complex of the Godthåb (now Nuuk) region of southern West Greenland, which show that discrete tectonic units recognized in the south of the region⁷ can be more widely identified. At least four terranes are recognized, each of which evolved separately until their juxtaposition in the late Archaean. This suggests that at least some Archaean high-grade gneiss complexes may resemble more recent orogenic belts, formed by plate-tectonic processes.

The field geology of the Nuuk region^{8,9} has been substantiated by much isotopic data¹⁰⁻¹⁵. This work distinguished a polygenetic older complex dominated by the early Archaean Amîtsoq gneisses, the Malene supracrustal rocks and a younger, polygenetic intrusive complex of quartzo-feldspathic gneisses, the Nûk gneisses. With the hypothesis of horizontal tectonics¹⁶ for thickening the crust, the region was established as one of major significance to the understanding of Archaean gneiss terrains. Although some aspects (for example, timing of igneous activity, metamorphism and deformation) have been debated¹⁷⁻²⁰ the broad model has stood essentially unaltered. A summary of the large data base has been presented elsewhere²⁰ and only references to key papers is made here.

New mapping in the south of the Nuuk region⁷, further field work in Ameralik²¹, new U-Pb zircon studies and reassessment of older work^{14,22} has shown that, contrary to previous suggestions¹⁶⁻²⁰, the rocks mapped as Nûk gneisses do not belong to one tectono-stratigraphic terrane mostly formed during the middle Archaean. Instead, the region comprises four distinct, separately evolved terranes (Table 1), the structural relationships of which are shown in Figs 1 and 2. The terranes now recognized in the Nuuk region are: (1) the Færingehavn terrane, dominated by early Archaean Amîtsoq gneisses that underwent granulite facies metamorphism about 3,600 Myr ago¹¹; (2) the Akia terrane, largely comprising 3,070-2,900-Myr-old gneisses, which in its north-western parts underwent granulite facies metamorphism about 3,000 Myr ago^{12,23}; (3) the Tasiarsuaq terrane, dominated by ~3,000-Myr-old rocks²⁰, which underwent granulite facies metamorphism during or after emplacement of the ~2,800-Myr-old Ilivertalik granite²², and which is probably continuous southwards into the Fiskensæstet region; and (4) the Tre Brødre terrane, dominated by the 2,800-2,750-Myr-old Ikkattoq gneisses, which has never undergone metamorphism above amphibolite facies^{7,21}. Each terrane contains supracrustal

Fig. 1 Geological sketchmap of the Nuuk region showing the broad distribution of the four terranes, the outcrop patterns of which are controlled by the F1 and F2 folds described. Some over-simplification of both geology and the terrane boundaries has been necessary to fit the scale. The section lines A-D show the line of the composite section in Fig. 2. Map compiled from: the Geological Survey of Greenland 1:500,000 scale map Sheet 2, Frederiksåbs Isblink-Søndre Strømfjord; unpublished Survey data; and our field observations.

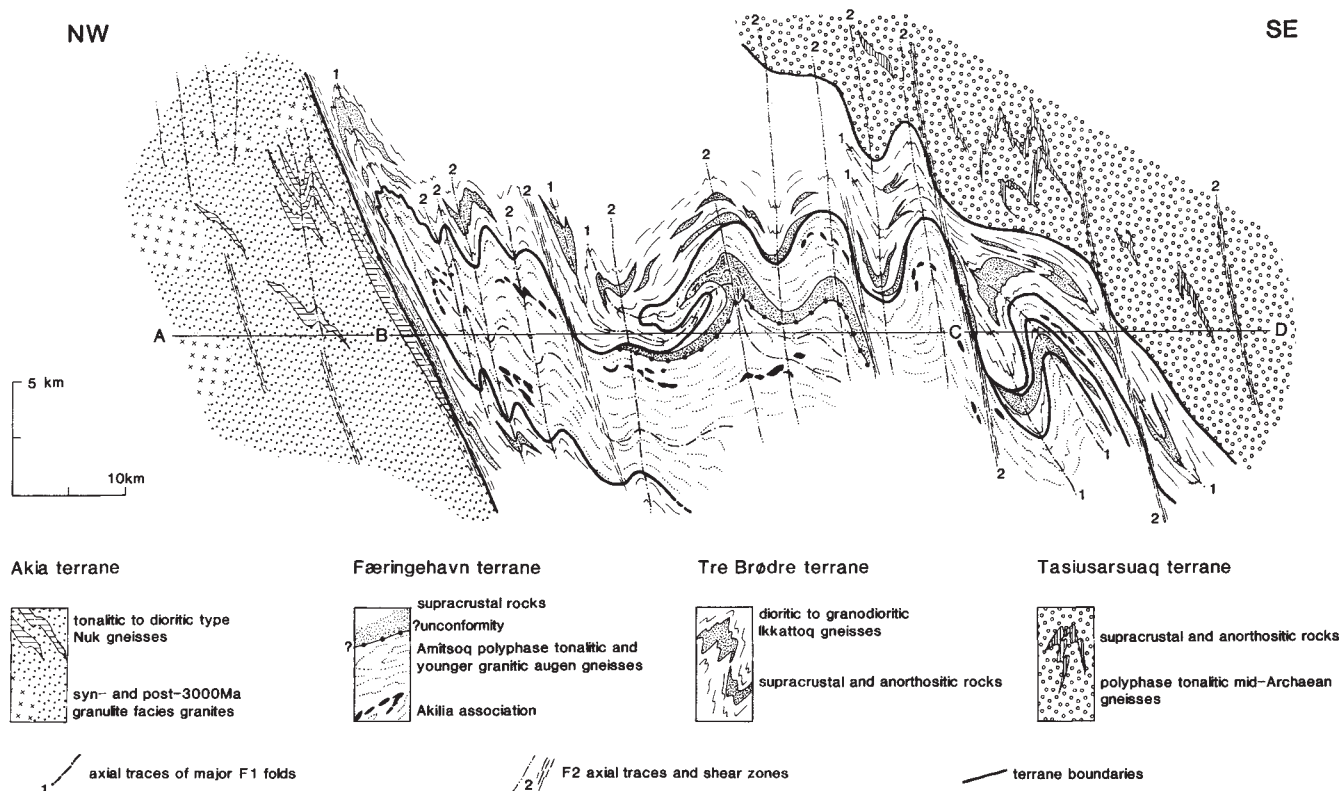
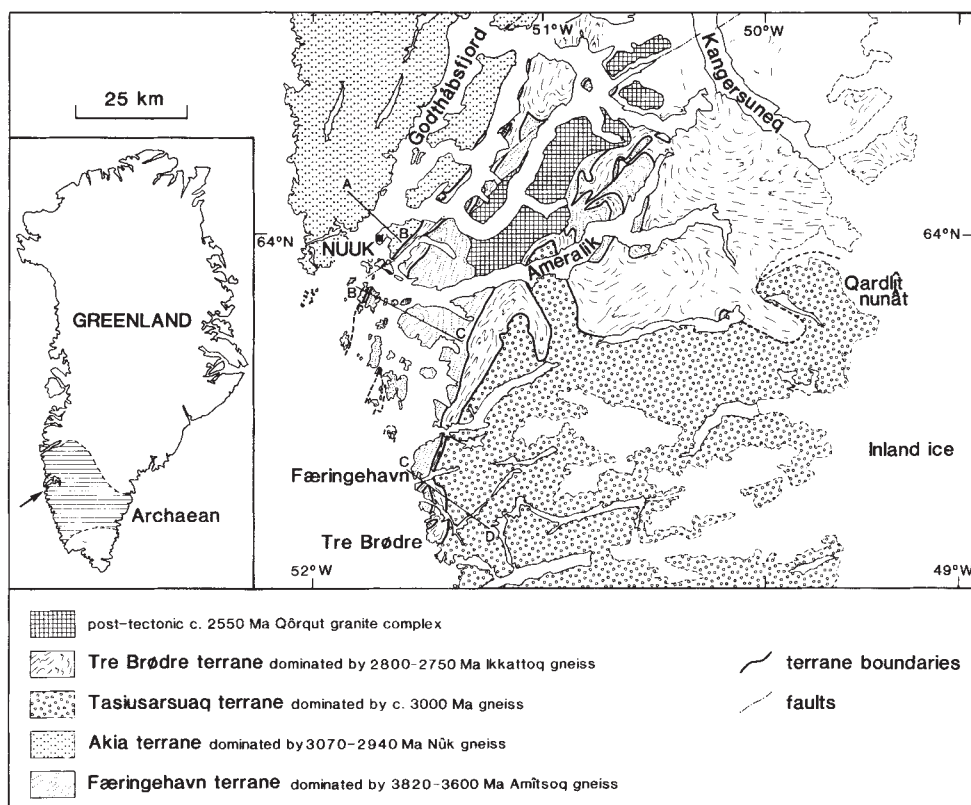


Fig. 2 Sketch cross-section of the Nuuk region (A-D in Fig. 1) showing the structure of the F1 nappe and associated isoclinal folds affecting the Færingehavn and Tre Brødre terranes. F2 upright basin and dome structures deform the sole thrust beneath the Tasiusarsuaq terrane and re-fold the F1 structures. The F2 axial traces are sub-parallel to the orientation of the Akia terrane boundary and are probably related to its arrival.

Table 1 New chronology and summary of the structural relationships of the four terranes found in the Godthåb region

Akia terrane	Færingehavn terrane	Tre Brødre terrane	Tasiusarsuaq terrane	Myr
Post-tectonic 2,550-Myr Qôrqu granite complex emplaced into the three southerly terranes.				2,500
Formation of "straight belts" with retrogression to epidote amphibolite and greenschist facies in NNE-trending zones.				
Emplacement of syn-deformational 2,610-2,690-Myr-old granitic sheets probably related to re-equilibration after arrival of the Akia terrane.				
Tectonic juxtaposition (assembly) of the Akia terrane against the other three terranes.				
F2 upright basin and domes giving rise to regional interference patterns and first folding of Qardlît nunât thrust (possibly related to arrival of Akia terrane).				
Arrival of the Tasiusarsuaq terrane, probably synchronous with the formation of F1 nappes in the Færingehavn and Tre Brødre terranes.				
Juxtaposition of the Tre Brødre and Færingehavn terranes.				
Granite emplacement and static retrogression of granulite facies assemblages at 2,900-3,000 Myr.		Intrusion of dioritic-granodioritic protoliths of the Ikkattoq gneisses, with deformation and metamorphism between 2,750-2,800 Myr.		2,750
		Emplacement of layered gabbro/anorthosite complex.	Granulite facies metamorphism and intrusion of the Ilivertalik granite at <i>circa</i> 2,800 Myr.	
		Formation of sediments and volcanic rocks. Perhaps on an Amîtsoq gneiss basement?	Intrusion of plutonic, predominantly tonalitic gneisses into a supracrustal sequence with layered gabbro/anorthosite complexes.	3,000
	Deformation; metamorphism (mainly static) to granulite facies at ~3,000 Myr.			
	Intrusion of plutonic protoliths of the voluminous tonalitic-dioritic type Nûk gneisses, 2,940-3,070 Myr, into supracrustal sequence dominated by basic rocks.			
	Ameralik dykes.	Fragments of included Amîtsoq gneiss complex and Akilia association.		3,250
	High-grade metamorphism to granulite facies and intrusion of Amîtsoq iron-rich suite at 3,600 Myr.			3,500
	Intrusion of protoliths of Amîtsoq grey gneisses. High-grade metamorphism and deformation. Pre-3,600 Myr.			3,750
	Formation of Akilia association >3,820 Myr.			

units originally mapped together as the Malene supracrustal rocks^{8,9}. Because of the individuality of each terrane it is probable that the supracrustal rocks are unrelated and are of different ages (Table 1).

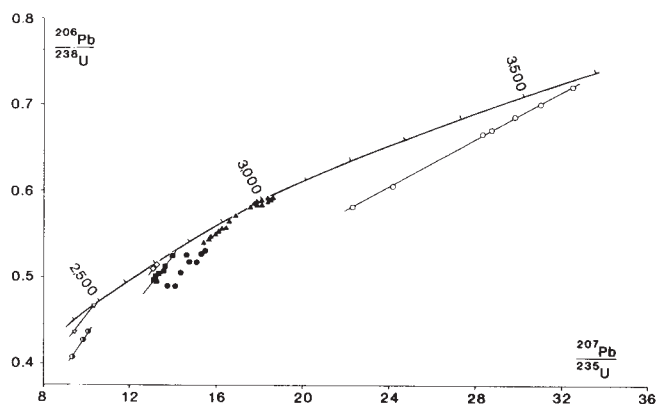
The four terranes are separated from each other by discordant zones up to 50 m wide, of mylonitic to ultra-mylonitic gneisses which contain fragments of the adjacent lithologies incorporated during tectonic transport. Preliminary studies on kinematic indicators in the bounding mylonites, which formed at different times, give inconsistent movement directions. This is probably due to subsequent deformation and associated metamorphism^{7,21}.

The areas now designated as the Akia and Tasiusarsuaq terranes were both originally mapped as including units of gneiss containing tabular fragments of basic rocks, which were interpreted as Amîtsoq gneisses cut by Ameralik dykes and migmatized by the igneous precursors of the Nûk gneisses^{8,9,20}. But extensive isotope studies in the Tasiusarsuaq terrane have failed to reveal any evidence that early Archaean rocks are present^{13,15,24,25}. This conflicts with the isotopic confirmation of the presence of early Archaean rocks in both the Færingehavn and Tre Brødre terranes^{7,10,11,13,15,21}, suggesting the possibility that the Akia and Tasiusarsuaq terranes may have been far from any early Archaean rocks during their initial evolution.

As the Tre Brødre terrane is in tectonic contact with the other three terranes (Fig. 1), tectonic assembly, which commenced with the Færingehavn and Tre Brødre terranes, began less than 2,750 Myr ago (Table 1). In some places the Ikkattoq gneisses contain volumetrically minor inclusions of all the lithological components of the Amîtsoq gneiss complex, which dominates the Færingehavn terrane. Furthermore, sheets of Ikkattoq-like gneiss are found locally within the Færingehavn terrane. Close to the contact between the two terranes, units of variably fuchsite quartzite occur. One of these contains a population of detrital zircons, derived exclusively from an early Archaean continental source about 3,630-3,890 Myr ago, that are similar to the Amîtsoq gneiss complex^{26,27}. The evidence suggests, therefore, that the Færingehavn and Tre Brødre terranes were in close proximity before the late Archaean thrusting.

The Færingehavn terrane lies in the core of a north-west-facing nappe (labelled F1), large-scale parasitic isoclinal folds of which deformed the contact with the Tre Brødre terrane (Fig. 2). The Tasiusarsuaq terrane, with its 2,800-Myr-old granulite facies assemblages, structurally overlies the amphibolite facies Tre Brødre terrane^{7,21}, and the boundary between them, not affected by F1 folding, has a much simpler outcrop pattern (Figs 1 and 2). Evidence from near Qardlît nunât suggests that the original boundary between these two terranes was a thrust, now modified

Fig. 3 Summary U-Pb diagram for zircons separated from gneisses from the Færingehavn and the Tre Brødre terranes in the Færingehavn area³⁰ (Fig. 1), the Akia terrane¹⁴ and the Tasiusarsuaq terrane²² and P. D. Kinny (personal communication), demonstrating the different ages of the rocks from each terrane. Note the late Archaean age of the Ikkattoq gneisses. Key to symbols: ○, Amîtsoq gneisses (Færingehavn terrane); ▲, Nûk gneisses (Akia terrane); ●, middle Archaean gneiss (Tasiusarsuaq terrane); ■, Ikkattoq gneisses (Tre Brødre terrane); ◇, composite diorite-granite sheet (Qârussuk dyke); ○, Sâtut gneisses, which cross-cut the boundary between the Færingehavn and Tre Brødre terranes and thus give the latest time by which assembly had taken place; ▽, undeformed granite pegmatite (probably associated with the Qôrquq granite complex, Table 1).



by later events, so that the possibility of a transcurrent component cannot be eliminated. It is possible that the F1 nappe structure developed in the already-assembled Færingehavn and Tre Brødre terranes as the Tasiusarsuaq terrane was thrust into position. Following this tectonic thickening of the crust, north-east-trending upright folds (labelled F2) developed, resulting in regional-scale interference patterns^{7,21,28,29}. These folds often have a sheath-like morphology and parts of the limbs may be replaced by steeply dipping shear zones (Fig. 2). The mylonitic boundary of the Akia terrane is now largely steep, trends north-north-east to north-east and cuts across the geology. The steepness is a function of later zones of intense deformation (Table 1), outside of which it dips north-west at moderate angles. Transpression during docking of the Akia terrane could account for the formation of the F2 folds, which have north-east-trending axial surfaces and decrease in amplitude south-eastwards away from the contact. The precise time by which terrane assembly was completed is uncertain, but it had taken place more than 2,600 Myr ago, before the formation of the late-tectonic granitoid sheets that occur in all four terranes^{20,30} (Fig. 3).

Amphibolite facies metamorphism associated with both the F1 and F2 folding³⁰ caused extensive modification of earlier mineral fabrics, formed within the individual terranes before assembly into their present configuration. The metamorphic assemblages observed are therefore polyphase, resulting from overprinting of relic granulite and amphibolite facies assemblages formed before assembly.

A summary of recent U-Pb dating³⁰ of zircons from the Færingehavn area (Fig. 1), with earlier data from the Akia and Tasiusarsuaq terranes^{14,20,22}, demonstrates that in each terrane the gneisses have distinct ages (Fig. 3). Most importantly, these data confirm the existence of a substantial unit of late Archaean rocks, the 2,800–2,750-Myr-old Ikkattoq gneisses, recognized from the new studies^{7,21,30}. Dating of the regionally extensive Ikkattoq gneisses demonstrates that sialic crustal accumulation in the Nuuk region occurred as a discrete episode in the late Archaean, earlier than assumed in previous syntheses^{16,17,19,20,31}. Formation of new sialic crust (as opposed to recycling by crustal re-melting) took place during at least three different periods in the Nuuk region: (1) between 3,820 and 3,750 Myr ago for the Amîtsoq gneisses in the Færingehavn terrane; (2) between 3,070 and 2,940 Myr ago in the Akia terrane and probably close to 3,000 Myr ago in the Tasiusarsuaq terrane; and (3) between 2,800 and 2,750 Myr ago in the Tre Brødre terrane. Final assembly of the region began only 2,750 Myr ago and was completed about 100 Myr later.

The grey gneisses that are the dominant components of Archaean gneiss complexes are of calc-alkaline affinity (see, for example, refs 31–33) and have similarities with magmas gener-

ated at modern destructive plate margins^{32–34}. It has been argued that these rocks imply an Archaean form of plate tectonics^{5,6,32–34}. The significant geochemical differences from modern calc-alkaline rocks are explained by the different thermal regimes that control melting in each case³⁵. Each of the terranes in the Nuuk region is dominated by gneisses of calc-alkaline affinity; units of low-potassium tholeiitic and pillowed basic rocks are widespread. These are interpreted³⁶ as remnants of oceanic-type crust which could have provided a suitable source material for the gneiss precursors.

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British bird species distributions and the energy theory

John R. G. Turner, Jack J. Lennon & Jane A. Lawrenson

Department of Genetics, University of Leeds, Leeds LS2 9JT, UK

The steep decline in the number of species from Tropic to Pole may possibly be explained by 'the weather', that is, the latitudinal decline in the input of solar energy. This explanation, often obvious to the lay person, is dismissed or even ignored by most ecologists¹⁻³. Only J. H. Brown and colleagues have proposed a direct 'species-energy' relationship⁴⁻⁶, an excellent scientific theory which implies simple tests that would refute the theory if it failed them. One such is the change in species numbers according to the seasonal fluctuation in the distribution of available energy: we will show that the varying distribution of small insectivorous birds in Britain in summer and winter corresponds well with the predictions of the species-energy theory; it also provides support for the theoretical model which explains it^{6,7}. We find no support for the alternative 'historical' and 'habitat' theories.

The species-energy hypothesis predicts that species-richness will correlate with available solar energy (usually measured by temperature and sunshine hours): such a correlation has been demonstrated, either worldwide or locally, for birds⁶, butterflies and moths (Great Britain)⁷ and terrestrial vertebrates (Soviet Union)⁸; plant diversity^{6,9} and tree diversity (North America)¹⁰ correlate with temperature and rainfall, or with evapotranspiration, which is a function of these two. But this is not a real test of the theory, as both species-richness and overall annual energy are known to decline from Tropic to Pole; the correlation is therefore to be expected, regardless of any further explanation, and may not indicate a causal connection. Other theories¹¹⁻¹⁴ that account for the species-gradient are also consistent with this correlation.

A more rigorous test can however be performed: the correlation should exist only during those parts of the year when organisms are actively absorbing energy, and should disappear if energy absorption ceases (summer visitors should show no relationship to winter energy), or even become negative if irreplaceable energy is being expended, as when an insect hibernates. Thus while all organisms are expected to show a positive correlation during the growing season, different correlations should appear during the winter, according to where and how that season is passed.

We have therefore examined the distribution of 54 species of birds in Great Britain (Fig. 1) during the breeding season¹⁵ and during the three most inhospitable months of the winter¹⁶. The birds are divided into four groups: year-round residents, that pass both seasons in the country (split into their summer and winter distributions), summer visitors that leave the country in winter, and winter visitors that only over-winter in Britain (this includes replacing migrants which are present also in the summer, but which are represented by two independent populations in the two seasons). The species-richness of these four groups has been estimated in 75 quadrats of 400 km² for which we have direct readings of temperature and sunshine^{17,18} (Fig. 1).

Regression analysis (Table 1) shows relationships that are almost exactly those predicted by the species-energy theory: the diversity of summer visitors is correlated with the summer temperature only, and of winter visitors with the winter temperature only; the distribution of the year-round residents is correlated with both summer and winter temperatures, both in their summer and their winter ranges (manifested in the summer as a correlation with the 'continentality', or range of temperature between summer and winter, and during the winter as a correlation with both the summer and the winter temperature).

The climatic variables are not merely a replacement for the

Table 1 Multiple regression analysis of numbers of species of small insectivorous birds against climatic and other environmental variables

Variable	Regression	Degrees of freedom	F value*
Residents—summer breeding distribution			
Coefficient of determination (R^2)†	0.56	2/72	46
Intercept	28.49	—	—
Temperature range	-0.74	1/72	21
Latitude	-0.063	1/72	91
Residents—winter distribution			
Coefficient of determination (R^2)‡	0.75	3/71	69
Intercept	-3.96	—	—
Summer temperature	2.53	1/71	95
Winter temperature	2.17	1/71	17
Spring temperature	-3.32	1/71	19
Summer visitors—breeding distribution			
Coefficient of determination (R^2)§	0.53	2/72	40
Intercept	-11.56	—	—
Summer temperature	2.28	1/72	17
Altitude	0.015	1/72	79
Winter visitors			
Coefficient of determination (R^2)¶	0.68	3/71	51
Intercept	-4.19	—	—
Winter temperature	0.66	1/71	30
Autumn sunshine	0.026	1/71	29
Urbanization	1.71	1/71	16

Analysis is by backward, single-step multiple regression (removing insignificant variables one at a time, in inverse order of their F values), using the SAS computer-routine. The significance level is set at $1 - (0.95)^{1/n}$, where n is the number of regressors entered in the analysis, giving a 5% probability in each analysis that a random regressor is included by chance alone. The dependent variables are the numbers of small insectivorous bird species found in each quadrat (Fig. 1). The total set of independent variables, all entered at the start of the analysis is: (1) climate, that is temperature and sunshine in spring, summer, autumn and winter, and temperature range (continentality) (Fig. 1 for further definitions); (2) habitats, that is the proportion of squares in the quadrat possessing heathlands, moorlands, sessile oakwoods, limestone (solid geology), and large (>0.5 hectare) bodies of fresh water, the total number of these habitat classes recorded in the quadrat (data from ref. 24), the proportion of the quadrat occupied by urban development i.e. covered by 1/1250 Ordnance Survey plans³⁶, the narrowest width of the country taken in a straight line coast to coast passing through the centre of the quadrat (to test for 'peninsular effect'), and the approximate time for which the area has been free of glacial ice-sheets²³; (3) physical, that is latitude and longitude (in tens of kilometres on the transverse Mercator National Grid³⁶) and altitude of the temperature-recorder in metres above sea-level¹⁸. Values of R^2 for significant subsets of regressors in independent analyses (without the climate variables) are (i) from all habitat variables: † 0.26; ‡ 0.44; § 0.19; ¶ 0.44; (ii) from all physical variables: † 0.43; ‡ 0.72; § 0.49; ¶ 0.54. An equally satisfactory explanation ($R^2 = 0.74$) is given by the three regressors summer temperature (+ve), temperature range (-ve) and spring temperature (-ve).

* Probabilities for all F values are less than 10^{-4} , and can all be regarded as highly significant.

latitudinal prediction of diversity: they give larger coefficients of determination than are given by latitude, longitude and altitude alone, which are usually rejected from the analysis if they are included alongside the climate. The anomalous negative correlation of one group with the spring temperature, and of another group positively with altitude require further investigation, but appear to be 'spoiler' effects, characteristic of multiple regressions, as they both reverse the signs of the simple regressions.

An older, more popular and complex 'structural' alternative to the energy hypothesis¹¹ proposes that it is not the overall

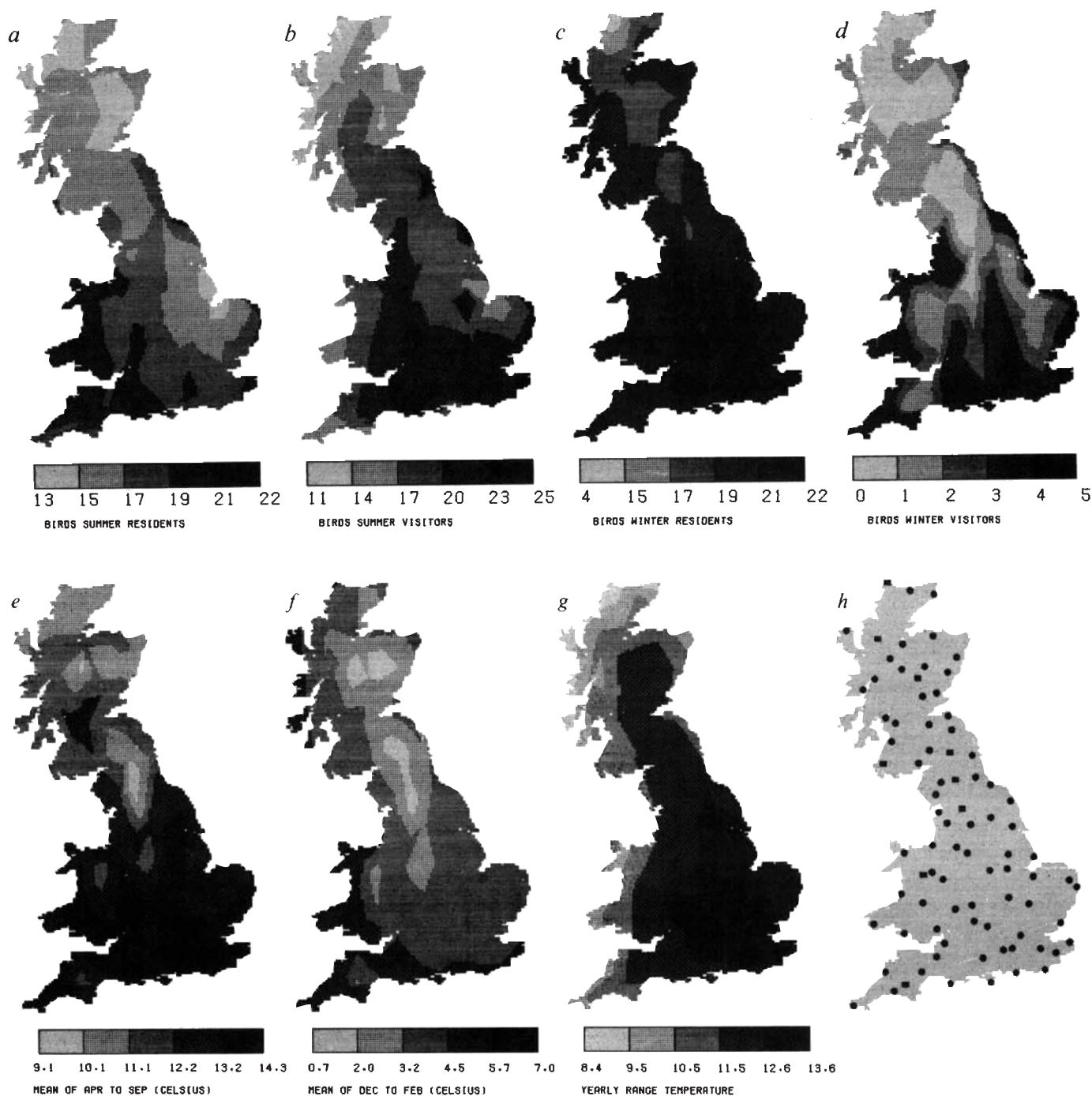


Fig. 1 *a-d*, Distribution of species-richness of small insectivorous birds: *a*, resident species (summer), *b*, summer visitors, *c*, resident species (winter), *d*, winter visitors and replacing migrants. *e-g*, Distribution of three of the significant environmental variables: *e*, summer temperature; *f*, winter temperature; *g*, temperature range; *h*, locations of quadrats, each centred on a climate recording station^{17,18}, with (squares) 9 additional stations used in interpolations but not in the regression analyses. Temperatures are not reduced to sea-level. Small insectivorous bird means not more than 20 cm in length and 60 g³⁰ (smaller than a starling), feeding on invertebrates for much of the year. The species are: residents—lesser spotted woodpecker, skylark, meadow pipit, rock/water pipit, grey wagtail, pied [white] wagtail, dipper, wren, dunnoek, robin, stonechat, Cetti's warbler, Dartford warbler, goldcrest, bearded tit, long-tailed tit, marsh tit, willow tit, crested tit, coal tit, blue tit, great tit, nuthatch, treecreeper; summer visitors (including summer populations of replacing migrants)—wryneck, woodlark, swift, wheatear, whinchat, redstart, black redstart, nightingale, grasshopper warbler, Savi's warbler, reed warbler, marsh warbler, sedge warbler, garden warbler, whitethroat, lesser whitethroat, willow warbler, chiffchaff, wood warbler, blackcap, firecrest, spotted flycatcher, pied flycatcher, tree pipit, yellow wagtail, red-backed shrike, swallow, house martin, sand martin; winter visitors (with replacing migrants)—shorelark, woodlark, black redstart, chiffchaff, blackcap, firecrest. For translations of names into Latin and other European languages, see ref. 31. Species-richness is the number of species recorded in each quadrat during the winters (mid-November to February) of 1981–84 (for winter distributions)¹⁶ and certainly or probably breeding in the quadrat in the years 1968–71 (for summer distributions)¹⁵. All climatic variables are 30-yr means or totals for the period 1941–70^{17,18}, except for temperature at 11 stations and sunshine at six stations, which is the 30-yr average for 1931–60^{32,33} or 1921–50^{34,35} adjusted to the period 1941–70 by 'averaging'^{17,18} to the nearest comparable station. 'Summer' is the growing season April–September, 'winter' the three months December–February, and 'spring' and 'autumn' the remaining months of March, October and November. Temperature is the average of the daily mean for the whole period in Celsius, sunshine is the total hours for the period in question. Temperature range is the difference between the means of the warmest and coolest months (not always July and January). The distribution of climate recording stations is based on the grid in ref. 37, and excludes offshore islands. Each quadrat consists of the four 10 km squares of the National Grid surrounding the 10 km intersection closest to the climate station; for sites on the coast, adjacent 10 km squares have been added to the quadrat to bring the total land area as near as possible to 400 km². The contours of the variables, *a-g*, are numerically interpolated between these points, with the total range for each variable divided into five intervals.

energy supply that determines species-richness, but its seasonal constancy: a steadier supply permits finer ecological structuring. The present findings are consistent with this, as the species-richness of the resident birds does correlate negatively with the range of temperature between the warmest and coldest months (Table 1). However, our previous findings for lepidoptera⁷ which are more diverse with warm summers but with cold winters, are not consistent with this view. They are consistent with the energy theory, in that diapausing insects expend but cannot consume energy, and may therefore be adversely affected by mild winters which raise their metabolic rate and deplete their reserves.

The 'historical' theory which explains diversity gradients¹⁴ by the pattern of recolonization and the maturation of ecosystems after the retreat of the glaciers, is widely accepted¹⁹⁻²², but is of no assistance with the present distributions: the time for which our quadrats have been free of glacial ice²³ is invariably rejected from the regressions (Table 1). Alternatively, most naturalists notice a decline in habitat richness from south to north in Britain, which might explain the species-diversity gradient. We have therefore examined the influence of the distribution of six special habitats (Table 1), five of them thought to be especially rich in bird species²⁴, as well as the total number of these habitats per quadrat. These variables on their own always give a much lower coefficient of determination than the temperature variables, and when combined with them, fail to remain in the set of significant regressors. These aspects of the habitat at least do not predict species-richness among birds. The exception is urbanization, which partly predicts the diversity of winter visitors, probably on account of the preference of two species (chiffchaff and blackcap) for industrial warmth and suburbs¹⁶.

A simple stochastic theory can explain the relationship between energy and species-richness: given the observed constant turnover (colonization and extinction) of species in any one area^{25,26} regions where populations are larger will have more species at equilibrium, as smaller populations become extinct more often^{6,7,27}. Greater energy supplies might increase populations of birds indirectly, through overall productivity, or directly, on account of the lower metabolic energy required to maintain body functions when the air is warmer: cold weather kills birds²⁶. Our regressions provide some support for this view: if birds were merely seeking immediately favourable weather, resident species should correlate only with the current season: the fact that both their winter and their summer distributions correlate with both the winter and summer weather, suggests that the climate is influencing their ability to maintain viable populations round the year.

If the direct energy budget of the animals is significant, warm-blooded endotherms will show only weak correlations with sunshine. Birds do not use direct sunlight for temperature control, but only for feather conditioning²⁹: the absence of correlation with sunshine hours (with one exception), therefore suggests that the species-energy relationship does involve the organisms' own thermal budget. By contrast, lepidoptera, being ectotherms which regulate their temperature by basking in sunlight or by living in the boundary layer of air that is heated by the sun, do show correlations both with temperature and sunshine⁷.

The diversity of these birds therefore shows the varying correlations with seasonal climates that are predicted by the species-energy theory⁵, which needs to be considered seriously alongside the other structural¹¹⁻¹³ and historical¹⁹⁻²² theories that explain the Tropic-Polar gradient, and which, as far as direct testing has been possible, are not supported by the present data.

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Spatial clumping of sexually receptive females induces space sharing among male voles

Rolf Anker Ims

Department of Biology, Division of Zoology, University of Oslo,
PO Box 1050, Blindern, N-0316 Oslo 3, Norway

The spatial organization of individuals in populations (their spacing system) can be highly variable even among populations of the same species¹. As spacing systems have important consequences for ecological processes such as population regulation, competition and mating systems^{2,3}, there have been many attempts to explore factors that may cause this variation. For mammals, it has been argued that the spatial distribution of sexually receptive females is the most important factor determining the spacing system of males, whereas habitat characteristics are most important to females⁴⁻⁶. This has been difficult to test experimentally as it requires manipulations of the spatial distribution of the opposite sex without changing other properties of the environment⁷. Here, I present a novel experimental procedure that can achieve this and demonstrate that the spatial distribution of the opposite sex in a population of voles is indeed an important determinant of the spacing system of males, but not of females. However, the effects on males are different from those predicted by many theoretical studies.

In small mammals, male home ranges are either large and overlap extensively with those of other males or they are small and non-overlapping (called territories)^{5,6}. Female spacing systems also show considerable variation^{5,6,8}. According to conventional wisdom the spacing system of males is determined by the

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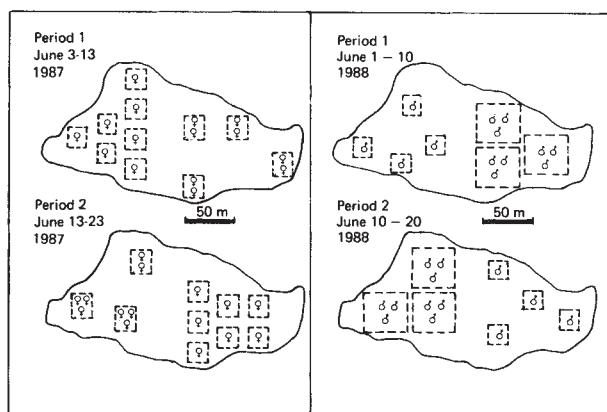


Fig. 1 The dispersion pattern of SMHR and SFHR on the island during the two experiments. The number of female and male symbols inside each dashed square is the number of caged individuals per simulated home range. Each of the two time periods constitutes a replica of two separate dispersion patterns of the caged sex which are defined by the spatial arrangement of simulated home ranges in each half of the island. The half of the island with a few widely separated SFHRs, containing groups of 2-3 females, mimics a situation where groups of females have extensively overlapping home ranges (referred to as clumped dispersion pattern). The other half of the island has single females on uniformly distributed SFHRs and represents a situation of female territoriality (referred to as dispersed female dispersion). In the experiment with caged males, the half of the island with groups of three males sharing one large SMHR mimics a situation where males have large, extensively overlapping home ranges. The other half of the island has single males on exclusive, small SMHRs and represents a situation of territorial males. These dispersion patterns can be found among free-living voles^{6,13,17}. Because the sexual status of females can affect the spatial distribution of males¹⁷ I monitored sexual status of caged females by examining vaginal smears^{22,23}. I held the proportion of oestrus females constant (at 50%) for both dispersion patterns by exchanging females between SFHRs and a colony of caged females separated from the experimental plot. The position of oestrus females were changed to ensure that all SFHRs had an oestrus female at least once during each time period. Between the introduction of animals onto the island and the start of the experiment, free-ranging individuals had a 10-day adjustment period in which cages were arranged in a random pattern over the whole island.

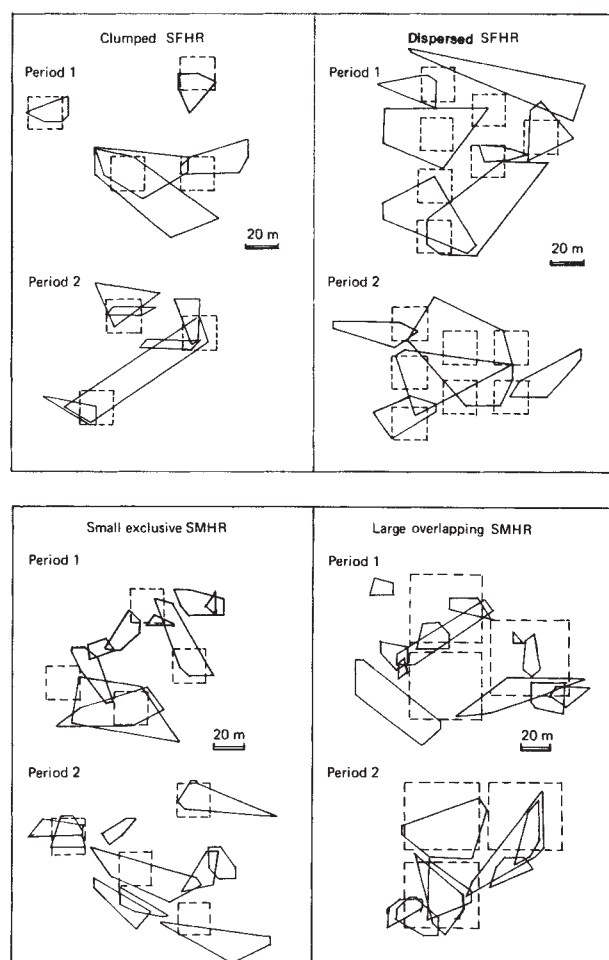


Fig. 2 Home ranges of radio-collared (Biotrack, Dorset, England), free-ranging males and females represented by minimum convex polygons (solid lines) in relation to different dispersion patterns of simulated female and male home ranges (dashed squares). Minimum convex polygons were drawn on the basis of coordinate points obtained by radiotelemetry. Radiotelemetry was not started until three days after rearrangement of the dispersion pattern of simulated home ranges to allow free-ranging individuals to adjust to the new spatial distribution of caged animals. Thereafter radiotracking was performed during the remaining seven days of each of the two periods. Each radio-collared vole was located 4-6 times per day, usually in the evening when they were most active. The different numbers of home ranges in the two periods are caused by the loss of radio-collared voles during the experiments. No male home range included any area which covered the different female dispersion patterns simultaneously, whereas one female home range overlapped with SMHRs from different male dispersion patterns.

spacing system of females, but not vice versa. Furthermore, territoriality is predicted to provide the optimal use of space for males when females are spatially clumped, but not when they are dispersed^{6,9,10}. However, theory far outstrips data regarding determinants of animal spacing systems^{7,11,12}.

I selected the grey-sided vole, *Clethrionomys rufocanus*, as the experimental species because it has a flexible spacing system¹³. The study consisted of two separate experiments. In the first, the spatial distribution of females was manipulated (June 1987); in the second it was the distribution of males (June 1988). The experimental populations originated from a laboratory colony of sexually mature animals of similar age that were introduced into the experimental plot: a two-hectare wooded island in south-east Norway. Laboratory animals were used to eliminate any effects of previous site tenacity, origin and age differences. To control for the spatial distribution of one of the sexes independently of habitat parameters, either males or females were kept in small wire-mesh cages. The cages, containing one individual each, were allocated to separate square areas which correspond to the home-range size of free-ranging animals (Fig. 1). I have called these SFHR or SMHR (simulated female or male home ranges). Each half of the island got a separate spatial arrangement of SFHR/SMHR to mimic the extremes of different dispersion patterns found among free-ranging animals (Fig. 1). This was replicated once during both experiments. Movements

and scent marking by caged animals within SFHR/SMHR were simulated by moving the cages to new, randomly selected, positions once each day. Space use of the free-ranging sex was monitored using radiotelemetry during the experiment.

The validity of this experimental system as an adequate model of a natural population relies upon the assumption that free-ranging animals perceived the opposite sex as 'normal mates' despite the physical separation created by the wire mesh. The following evidence indicates that this assumption was valid. Males were located close (nearer than 2.5 m) to caged females 14 times as often, and females close to caged males 2.4 times as often, than would have been expected if males and females moved around independently of the opposite sex (males were significantly more often close to females than females to males: $\chi^2 = 929$, $P < 0.001$). Further, males were found more often close

to females with oestrous vaginal smears (sexually receptive) than females with anoestrous smears ($\chi^2 = 4.8$, $P < 0.05$). Male home ranges overlapped with SFHRs to a greater extent (Fig. 2) than would have been expected if there was no spatial association between them; on average 42% of male home ranges overlapped with SFHRs although SFHRs covered less than 25% of the total island area ($t = 4.1$, $P < 0.001$). By contrast, female home ranges are random with respect to SMHRs (Fig. 2); percentage overlap between female home ranges and SMHRs (39%) was not different from the expected 38% ($t = 0.2$, not significant). This result together with the fact that females were located more often close to males than expected from random (2.4 times, $\chi^2 = 37.1$, $P < 0.0001$) indicate that a female's position within her home range was affected by the presence of caged males, but that the position of her home range was unaffected by the opposite sex. Males tended to aggregate on SFHRs that contained groups of females (Fig. 2), but not where females were spatially dispersed. As a consequence of this, males shared significantly more space on SFHRs when females were clumped than when females were spatially dispersed (Table 1). Overall, males avoided sharing space, whereas female home ranges overlapped more than expected: ($n - 1$) male home ranges covered on average 35% of the island area whereas the home range of a male on average overlapped 22% with other male home ranges ($t = 2.3$, $P < 0.05$), whereas the corresponding figures for females were 25% and 37% ($t = 2.5$, $P < 0.05$).

Three main conclusions can be drawn from the results presented here. First, the spacing of female home ranges in *C. rufocanus* is not affected by the spatial distribution of males. This result supports the conclusions of earlier experimental^{13,14} and theoretical studies⁶ showing that habitat characteristics (for example, food distribution) are the main determinants of the spacing system of females. Second, the spatial distribution of males reflects the spatial distribution of receptive females. This result is consistent with the prediction that receptive mates, rather than food or habitat characteristics, are the key factors determining male fitness¹⁵⁻¹⁷. Third, in contrast to the predictions of theoretical studies, spatial clumping of females was not sufficient to induce territoriality in males^{4,7}. On the contrary, I found most space sharing among males when females were clumped. Elsewhere I have shown that *C. rufocanus* males have the capacity to switch to territoriality when the density of competitors is low¹³. Sometimes an increased intruder pressure may be the inevitable consequence of spatial clumping of females because competitors are attracted to patches where the critical resource is abundant^{18,19}. Hence, spatially clumped females may in fact be more difficult to monopolize than females that are spatially dispersed. In particular, this may be true for small species like

C. rufocanus which live in a complex habitat, where the probability of detecting intruders is low²⁰. These results caution against making over-general predictions about determinants of male spacing systems. Different species may respond differently to equivalent conditions depending on other features of their biology. Field experiments of the kind reported here can be very useful in revealing these important species-specific features.

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Fully effective contraception in male and female guinea pigs immunized with the sperm protein PH-20

Paul Primakoff, William Lathrop, Laura Woolman, Ann Cowan & Diana Myles

Department of Physiology, University of Connecticut Health Center, Farmington, Connecticut, Connecticut 06032, USA

Immunization of male and female animals with extracts of whole sperm cells is known to cause infertility¹⁻⁵. Also, men and women who spontaneously produce antisperm antibodies are infertile but otherwise healthy⁶. Although the critical sperm antigens are unknown, these observations have led to the proposal that sperm proteins might be useful in the development of a contraceptive vaccine⁷⁻⁹. The guinea pig sperm surface protein PH-20 is essential in sperm adhesion to the extracellular coat (*zona pellucida*) of the egg, a necessary initial step in fertilization¹⁰. Here, we report that 100% effective contraception was obtained in male and female guinea pigs immunized with PH-20. Antisera from immunized females had high titres, specifically recognized PH-20 in sperm extracts, and blocked sperm adhesion to the egg *zona pellucida* *in vitro*. The contraceptive effect was long-lasting and reversible: immunized females, mated at intervals of six to fifteen months after immunization, progressively regained fertility.

Table 1 Characteristics of space use of males in relation to the dispersion pattern of SFHRs

Male space-use characteristics	Female dispersion pattern		Wilks' λ †
	Clumped (N = 11)	Dispersed (N = 13)	
% Overlap with males on SFHR*	55 ± 12	18 ± 6	0.72
% Overlap with males outside SFHR*	10 ± 6	17 ± 7	0.56
% Overlap with SFHR	47 ± 6	36 ± 5	0.47
Home range size of males (m ²)	605 ± 183	898 ± 175	0.43

Values are presented as mean ± standard error of the mean for pooled data from the two periods (Fig. 2). All estimates are based on minimum convex polygons of home ranges drawn on the basis of coordinate points obtained from radiotelemetry (Fig. 2). Variables are listed in the order in which they were entered into a step-wise discriminant function analysis²¹, used to determine which space-use characteristics were significantly affected by female dispersion pattern.

* Significant variables.

† Wilks' λ is an inverse measure of the discriminating power of the discriminant function after the corresponding variable entered the function.

An integral membrane protein, PH-20, with a relative molecular mass (M_r) of 64,000, identified by monoclonal antibodies (mAbs), is present on both the plasma membrane and inner acrosomal membrane of guinea pig sperm¹⁰⁻¹³. To determine whether immunization with affinity-purified PH-20 (ref. 13) would affect fertility, four female guinea pigs were each immunized with different PH-20 doses (10, 20, 30 and 50 μ g) which were given in a first injection and again in a second injection one month later. Control animals received injections lacking PH-20 at the same times. Approximately two months after the initial injection, control-injected and PH-20-injected females were housed with the same males and allowed to mate. None of the four PH-20-immunized females had litters. Twelve of 13 (92%) of control animals had litters; the 13 controls gave birth to an average of 3.5 progeny per litter (Table 1, group 1).

A second group of 21 females were immunized with PH-20 (5, 10 and 20 μ g) using the same schedule as the first group. None of these animals had litters, although 22 out of the 23 (96%) control animals did (Table 1). In total, 34 out of the 36 (94%) control-injected animals had litters and the 36 controls gave birth to an average of 3.3 young. Based on this average, the 25 PH-20 immunized females would have been expected, if fertile, to give birth to 82 progeny; that there were none indicates 100% effective contraception in these animals (Table 1).

Antisera, obtained from injected females one week before

mating, were titred in a solid-phase assay using a detergent extract of whole sperm as antigen. Antisera from the infertile females showed high binding levels that began to decrease significantly at dilutions greater than 10^{-4} and remained measurable even at dilutions of 10^{-8} (Table 2).

Like anti-PH-20 mAbs¹⁰, the anti-PH-20 polyclonal sera from the infertile females blocked sperm binding to the egg *zona pellucida in vitro*. The relative inhibitory potency of the antiserum provided a second, perhaps more physiologically significant, measure of antiserum titres. In the *in vitro* sperm-zona binding assay¹⁰, antisera from the infertile females inhibited sperm-zona binding by 90-94% at a 10^{-2} or 10^{-3} dilution. The extent of inhibition decreased at 10^{-4} dilution, but remained substantial even at 10^{-5} dilution (Table 2).

The antisera from the infertile, PH-20-immunized females specifically recognized the PH-20 protein. The immunofluorescence-staining patterns obtained with sera (10^{-3} dilution) from females immunized with 5-50 μ g PH-20 were the same as those previously reported with anti-PH-20 mAbs¹⁰⁻¹²: surface-staining was restricted to the posterior head region on the plasma membrane of acrosome-intact sperm and to the inner acrosomal membrane of acrosome-reacted sperm. Sera from control-injected animals showed no fluorescent staining of sperm. In addition, antisera from infertile females recognized a single band in immunoblots of SDS extracts of whole sperm (Fig. 1,

Table 1 Contraceptive effect of PH-20 immunization

	Amount of PH-20 injected (μ g)	No. of animals	No. with litters (% with litters)	No. of progeny	Average no. progeny in control
Group 1 females	50	1	0	0	
	30	1	0	0	
	20	1	0	0	
	10	1	0	0	
	0 (control)	13	12 (92%)	46	3.5
Group 2 females	20	14	0	0	
	10	4	0	0	
	5	3	0	0	
	0 (control)	23	22 (96%)	74	3.2
Total PH-20 immunized females		25	0 (0%)	0	
Total control females		36	34 (94%)	120	3.3
Females with fetuses					
Mated females					
Group 3 males	50	1	0/2	0	
	30	1	0/2	0	
	20	1	0/2	0	
	10	1	0/2	0	
	5	1	0/2	0	
	2.5	1	0/2	0	
	0 (control)	7	14/14	62	4.4
Total PH-20 immunized males		6	0/12 (0%)		
Total control males		7	14/14 (100%)		

Female or male Hartley guinea pigs (about 300 g at the time of the first injection) received two injections of the stated amount of PH-20. PH-20, purified from sperm by mAb-affinity chromatography, showed no detectable contaminants using silver-staining of high loads (5 μ g) of protein on SDS gels¹³. Purity of each PH-20 preparation used for immunization of females or males was verified by SDS polyacrylamide gel electrophoresis and silver staining. The affinity-purified PH-20, in 0.375 ml phosphate-buffered saline (PBS) containing 3 mM octylglucoside (OG), was emulsified with 0.375 ml complete Freund's adjuvant (CFA). Each animal received 0.5 ml the emulsion subcutaneously in the back and 0.25 ml intramuscularly in a rear leg. About one month later the same amount of PH-20 in PBS and 3 mM OG, emulsified with incomplete Freund's adjuvant (IFA), was injected in the same sites in each animal. (A single exception was the male that received 30 μ g PH-20 and had only the primary injection.) Control females and males received the same injections on the same schedule and containing PBS and 3 mM OG and CFA or IFA, but lacking PH-20. To allow the injected females to mate, about two months after the initial injection they were housed with males for three weeks. Each cage contained one male (575-600 g), two or three PH-20 immunized females and from two to four control-injected females. After three weeks, the females were separated from the males, pregnant females had litters and progeny were counted. Control-injected females that failed to become pregnant had been in cages where the other controls did become pregnant, indicating that all the males mating with immunized females were fertile. To allow the injected males to mate, about two months after the initial injection, each injected male was housed with two females (about 600 g) for three weeks. The females and males were then separated and after an additional five weeks females were killed and fetuses counted.

lanes 1-5). This band co-migrated with purified PH-20, of relative molecular mass (M_r) 64,000 (64K) when immunoblotted with antiserum from the 50 μg -immunized female (Fig. 1, lane 7). The production of high levels of anti-PH-20 antibodies by immunized females indicates that the PH-20 protein may be a non-self antigen in females. To determine whether PH-20 is found on tissues other than sperm, we assayed a spectrum of other tissue types from female guinea pigs to see if they could bind the anti-PH-20 antiserum from the 50 μg -immunized female. The antiserum showed high binding to a sperm extract, but no binding was detectable to extracts of other tissues (Fig. 2). Addition of 50 $\mu\text{g ml}^{-1}$ sperm protein to 5 mg ml^{-1} tissue protein resulted in antiserum binding, indicating that the assay was sensitive enough to detect a 1% level of sperm extract in the tissue extract. We have also found that antiserum from the 50 μg -immunized female immunoblotted the PH-20 band in

sperm and testis extracts, but blots of the other tissues showed either no bands or bands that were also seen with control sera (data not shown). Thus, PH-20 may have one of the suggested theoretical advantages of sperm contraceptive immunogens^{5,9}: that it would not raise potential problems of auto-immunity when used in females.

To test for reversal of the contraceptive effect, the PH-20-immunized females were mated at intervals. The immunized animals progressively regained fertility: about six months after the initial injection, four of 23 tested (17%) were fertile; by 9-11 months 11 of 24 (46%) had regained fertility; and by 15 months, all four in the longest-studied (group 1) females had delivered litters. About a fourfold decrease (relative to initial antiserum titres) was found at six months for titres from group 1 females immunized with 10, 20 or 30 μg of PH-20. The 10- and 20 μg -immunized females were fertile at six months,

Table 2 Titres of antisera from females

Antiserum from animals injected with PH-20 (μg)		Antisera binding in radioimmune assay						
		^{125}I bound in microtitre well (c.p.m. $\times 10^{-2}$)						
		Antiserum dilution						
		10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
0 (control)		27	5	2	1	1	1	1
5		156	152	120	44	14	5	2
10		150	149	130	66	21	8	4
20		150	147	128	66	24	10	5
30		155	148	113	43	13	4	2
50		142	140	130	64	18	6	2

Antiserum from animals injected with PH-20 (μg)		Percentage inhibition of sperm-zona binding			
		Antiserum dilution			
		10^{-2}	10^{-3}	10^{-4}	10^{-5}
0 (control)		0	0		
5		92	94	84	58
10		94	91	63	48
20		94	94	67	26
30		92	92	57	50
50		92	90	72	37

Antiserum from animals injected with PH-20 (μg)		Antisera binding before first mating compared with second mating						
		^{125}I bound in microtitre well (c.p.m. $\times 10^{-2}$)						
		Antiserum dilution						
	Mating	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
0 (control)	1st	26	6	2	1	1	1	1
	2nd	25	5	2	1	1	1	1
10	1st infertile	144	145	110	40	9	5	3
	2nd fertile	139	128	59	13	4	4	2
20	1st infertile	142	139	105	33	10	6	3
	2nd fertile	144	129	59	16	6	4	3
30	1st infertile	155	144	109	42	14	6	3
	2nd infertile	152	132	60	14	5	4	2
50	1st infertile	142	144	125	59	18	8	3
	2nd infertile	145	139	104	37	12	8	3

Binding of antisera, obtained one week before the first mating and diluted as indicated, was measured in a radioactive solid-phase (microtitre plate) assay using an OG extract of live sperm as antigen and ^{125}I -protein A as the second reagent²⁶. Non-specific binding was blocked with 3% non-fat dry milk in PBS. Values shown were determined in duplicate in one experiment and were from one immunized female (50 μg and 30 μg) or the averages of four females (0 μg), two females (5 μg), one group 1 and two group 2 females (10 μg) and one group 1 and three group 2 females (20 μg). Group 2 females, immunized with 10 or 20 μg , had somewhat higher titres than group 1 females immunized with 10 or 20 μg . The ability of antisera, diluted as indicated, to inhibit sperm binding to the *zona pellucida* of guinea pig eggs was measured as previously described for anti-PH-20 mAbs¹⁰. Sperm, capacitated and allowed to acrosome-react in modified Tyrode's medium, were pre-incubated with the diluted antiserum for 15-20 min and then added to eggs in the continuing presence of diluted antiserum. After 30 min, eggs with bound sperm were washed into sperm-free drops, fixed, and the number of sperm bound to the *zona* counted in one plane of focus. The mean number of sperm per egg was 16.4 ± 3.3 in the absence of antiserum in the 13 experiments in the Table. Percentage inhibition is $(1.0 \text{ minus the number of sperm bound in the presence of antiserum/number of sperm bound in the absence of antiserum in the same experiment}) \times 100\%$. Antiserum from one female injected with each stated amount of PH-20 was tested one time at each dilution to determine percentage inhibition. Antiserum from the control-injected female was tested twice at 10^{-2} dilution. Binding of antisera, obtained one week before the first mating or one week before the second mating, diluted as indicated, was measured as in the top panel. Values shown are averages determined in duplicate in two experiments. Specific binding of c.p.m. at 10^{-3} dilution is maximal binding. Antiserum titre is defined as the dilution at which c.p.m. bound is half-maximal.

Fig. 1 Immunoblotting of an SDS-extract of whole cauda epididymal sperm and purified PH-20 protein with antisera from immunized females. Antisera were from females injected with the following amounts of PH-20: lane 1, 5 μ g; lane 2, 10 μ g; lane 3, 20 μ g; lane 4, 30 μ g; lane 5, 50 μ g; lane 6, 0 μ g (control-injected); lane 7 (50 μ g). The samples in lanes 1–6 were whole sperm extracts; the sample in lane 7 was purified PH-20. Sperm extracts were prepared by solubilizing fresh live cauda epididymal sperm in non-reducing SDS-polyacrylamide gel electrophoresis sample buffer, centrifuging and discarding the insoluble residue, and using the entire supernatant as sample. Immunoblots were performed as described²⁷ using antisera (1 in 3,000 dilution) and alkaline phosphatase conjugated-goat anti-guinea pig immunoglobulin G as second reagent. Relative molecular masses (K) of marker proteins are shown on the left.

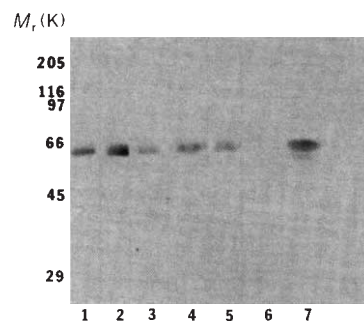
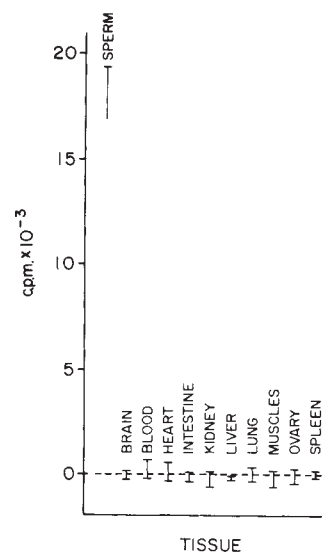


Fig. 2 Binding of antiserum (from female injected with 50 μ g PH-20) to extracts of a female's tissues, compared with binding to extract of sperm. Values shown are the range of four determinations for sperm and ovary and six determinations for other tissues; background binding obtained with control antiserum was subtracted from all values. Fresh tissues from a female and sperm from a male were disrupted in a Brinkmann Polytron in the presence of protease inhibitors¹³ and OG was added to 30 mM. Tissue extracts (at 5 mg per ml protein to ensure saturating amounts of antigen were present) and sperm extract (at 0.2 mg per ml protein) were allowed to bind to a microtitre plate overnight. Binding of antiserum (10^{-4} dilution) from the female injected with 50 μ g PH-20 and binding of antiserum (10^{-4} dilution) from a control-injected female were measured using 125 I-labelled protein A as second reagent.



whereas the 30 μ g-immunized female, with an identical titre, remained infertile (Table 2). This suggests that, when serum titres have fallen fourfold, any individual female has a certain probability of being either fertile or infertile.

Although immunization of males with a sperm protein raises the possibility of autoimmune disruption of testicular function, men with anti-sperm antibodies of natural occurrence or following vasectomy do not have autoimmune reactions in the testis^{6,14}. In an initial experiment to assess contraceptive effectiveness of PH-20-immunization in males, six male guinea pigs were immunized with 2.5–50 μ g PH-20; seven control males received injections without PH-20. About two months after the initial injection, each male was housed with two females for mating. None of the 12 females mated with the PH-20-immunized males carried fetuses; 14 out of 14 of the females mated with control males did (group 3, Table 1). Four of the six immunized males regained fertility by seven months after the initial injection and thus do not show the irreversible sterility associated with autoimmune orchitis.

Purified sperm proteins previously tested as contraceptive immunogens include the sperm enzymes hyaluronidase, acrosin and lactate dehydrogenase C-4. Immunization of female animals with these enzymes had either no effect on fertility^{15,16}, or partial effects on fertility^{17–19}, which were not large enough to make these proteins suitable for use as contraceptive agents^{8,9,20}. The high contraceptive effectiveness of PH-20 seems to depend on several of its specific properties, including its presence on the sperm surface, its strong immunogenicity and its essential role in fertilization. Human sperm are like guinea pig and certain other mammalian sperm in that they can bind to the *zona*

pellucida either before or after the acrosome reaction^{21–25}. Although the molecular details of these events are unknown with human gametes, a human functional analogue of PH-20 would be a candidate for an effective contraceptive immunogen.

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The *Caenorhabditis elegans* *lin-12* gene encodes a transmembrane protein with overall similarity to *Drosophila* Notch

John Yochem, Kathleen Weston*† & Iva Greenwald

Biology Department, Princeton University, Princeton, New Jersey 08544, USA

*MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

The *lin-12* gene seems to control certain binary decisions during *Caenorhabditis elegans* development, from genetic and anatomical studies of *lin-12* mutants that have either elevated or reduced levels of *lin-12* activity¹. We report here the complete DNA sequence of *lin-12*: 13.5 kilobases (kb) derived from genomic clones and 4.5 kb from complementary DNA clones. It is of interest that the predicted product is a putative transmembrane protein, given that many of the decisions controlled by *lin-12* activity require cell-cell interactions for the correct choice of cell fate²⁻⁵. In addition, the predicted *lin-12* product may be classified into several regions, based on amino acid sequence similarities to other proteins. These include extensive overall sequence similarity to the *Drosophila* Notch protein^{6,7}, which also is involved in cell-cell interactions that specify cell fate⁶⁻⁸; a repeated motif found⁹ in proteins encoded by the yeast cell-cycle control genes *cdc10* (*Schizosaccharomyces pombe*)¹⁰ and *SWI6* (*Saccharomyces cerevisiae*)⁹; and a repeated motif exemplified by epidermal growth factor, found in many mammalian proteins¹¹.

The *lin-12* locus had been cloned previously by transposon tagging and a 2.9 kb *Pst*I fragment including the insertion site had been sequenced, revealing that the fragment contained only part of the locus and that the predicted protein included an epidermal growth factor (EGF)-like repeating motif¹¹. Genomic restriction fragments (Fig. 1) flanking the original *Pst*I fragment were obtained and their sequences completely determined on both strands by M13 dideoxy sequencing^{12,13}. We also sequenced cDNA clones (Fig. 1). The cDNA clones together span 4.5 kb, in good agreement with the size of the major poly(A)⁺ RNA species detected on Northern blots¹¹ and with the coding region predicted from the genomic sequence analysis. Figure 1 summarizes the genomic and cDNA sequence analysis; only the cDNA sequence and limited regions of the genomic non-coding sequence are shown, but the complete genomic sequence data are available for inspection through the GenBank library or upon request.

The key finding of the sequence analysis is that *lin-12* appears to encode an integral membrane protein. This conclusion is drawn from two observations: there is a potential signal sequence¹⁴ at the amino terminus of the protein (the initial methionine is followed by an arginine and then 13 predominantly hydrophobic amino acids) and there is a potential membrane-spanning sequence¹⁵ internally (23 hydrophobic amino acids immediately followed by several basic amino acids).

The predicted *lin-12* product may be classified into four regions: the first three regions, which are amino-terminal to the putative membrane-spanning sequence, are probably extracellular. The first region includes 13 EGF-like (EGFL) repeats (Fig. 2a). The EGFL repeats are interrupted by a different cysteine-rich sequence (residues 62-108, Fig. 2a), encoded by open reading frames T and Y together. No similarity to other proteins has been found so far; although the amino terminal part of this sequence resembles an EGFL motif, the carboxy terminal part diverges from the EGFL consensus in the region which generally displays the highest conservation when EGFL motifs from different proteins are compared (see refs 11, 16, 17 for examples). The second region includes three tandem copies

of a cysteine-rich motif found in *lin-12* and *Notch* ('*lin-12/Notch* repeat', LNR1-3, Fig. 2b). The third region comprises the remainder of the putative extracellular domain. The fourth region consists of the putative intracellular domain, which is primarily composed of a series of six copies of the '*cdc10/SWI6*' repeated motif⁹ shown in Fig. 2c.

Previous genetic and developmental studies have indicated that *lin-12* determines the fates of certain bipotential cells that require cell-cell interactions for the correct specification of their fates¹⁻⁵. Thus, a membrane protein like *lin-12* might function as a receptor for an intercellular signal or as an intercellular signal. Sternberg¹⁸ proposes that *lin-12* acts as a receptor in vulval precursor cell determination. *lin-12* might also act as a receptor in gonadal cell determination. The fates of the gonadal cells Z1.ppp and Z4.aaa are naturally variable in wild-type: with equal probability, one becomes the anchor cell (AC) and the other becomes a ventral uterine precursor cell (VU) (ref. 19); the 'default' determination of an isolated Z1.ppp or Z4.aaa (all other gonadal cells ablated) is to the AC fate³. In *lin-12(0)* mutants, which by genetic criteria have no *lin-12* activity, both Z1.ppp and Z4.aaa become ACs; in *lin-12(d)* mutants, which by genetic criteria have elevated *lin-12* activity, both Z1.ppp and Z4.aaa become VUs (ref. 1). Thus, *lin-12* may encode a receptor for a signal emanating from the committed presumptive AC: in a *lin-12(0)* mutant, the receptor is missing, so both cells express the default state and become ACs; in a *lin-12(d)* mutant, the receptor may be active in the absence of a signal or hypersensitive to a low level of signal so both cells become VUs. Alternatively, *lin-12* might encode a 'regulative' signal, emanating from the committed presumptive AC: in a *lin-12(0)* mutant, the signal is absent, so both cells become ACs; in a *lin-12(d)* mutant, the signal may be more active or persistent so both cells become VUs. These possibilities, and others, may be distinguished by genetic mosaic analysis, which is underway (G. Seydoux, personal communication).

lin-12 displays extensive overall sequence similarity to *Notch*, a *Drosophila* gene that is required for a decision between neural and epidermal cell fates⁶⁻⁸ (Fig. 3). The extracellular region of both proteins contains tandem EGFL and LNR repeats in corresponding locations with respect to the putative membrane-spanning domain, and an intracellular domain containing six copies of the *cdc10/SWI6* motif. Although they share many structural features, *lin-12* and *Notch* may not be functionally equivalent, and the apparent differences may be significant. For example, there are 36 EGFL repeats in *Notch*, 13 in *lin-12*. The *Notch* EGFL repeats are more uniform and occur in one long tandem array; in *lin-12*, they are of irregular length and interrupted by the sequence encoded by open reading frames T and Y. The carboxy termini of the two proteins remain similar for about 32 amino acids following the *cdc10/SWI6* repeats, and then diverge substantially. Despite the similarity in overall structure, it may be that it is the amino acid differences that confer the specificity of the protein-protein interactions which are important in particular biological roles; for example, the receptor-binding domain of urokinase is its EGFL motif, but EGF does not compete for the binding of urokinase to its receptor *in vitro*²⁰, and the different EGFL repeats in *Notch* may have different functions^{21,22}. However, the remarkable similarity of *lin-12* and *Notch*, two genes involved in decisions of cell fate in distant phyla, suggests that proteins of this general structure may be ubiquitous and involved in other decisions of cell fate.

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† Present address: Hooper Foundation, University of California, San Francisco, California 94143, USA.

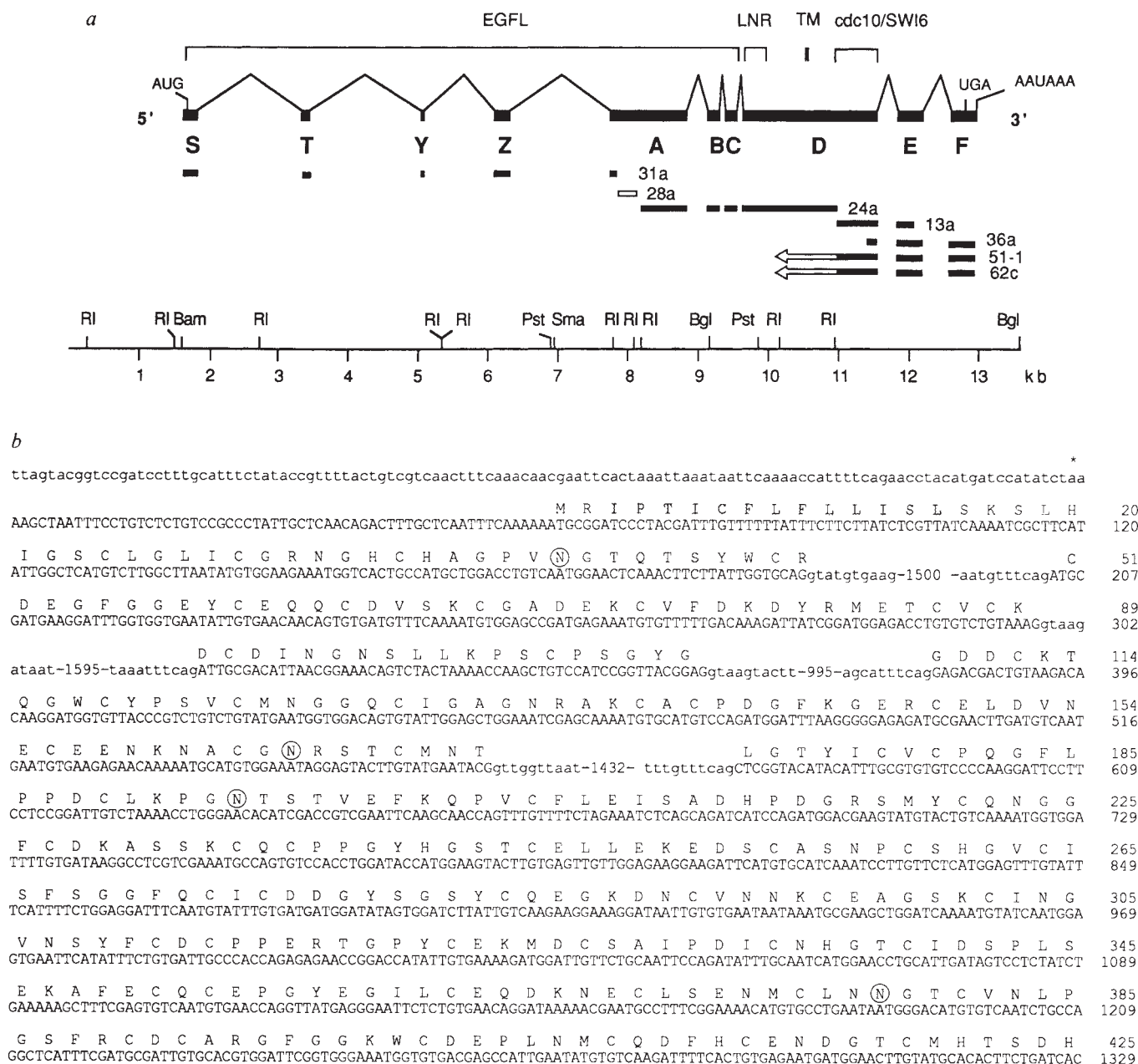


Fig. 1 DNA sequence and predicted protein product of the *lin-12* gene. **a**, The structure of the *lin-12* coding region deduced from the sequence of genomic and cDNA clones. The positions of the cDNA clones are indicated above a partial restriction enzyme map showing relevant *Bam*HI, *Bgl*II, *Pst*I and *Sma*I sites and all *Eco*RI sites. Filled areas indicate the regions of the cDNA clones that were sequenced. All *Eco*RI sites are shown because many of the cDNA clones terminated at *Eco*RI sites. The regions encoding EGF-like repeats (EGFL), a cysteine-rich repeat also found in *Notch* (LNR), a potential transmembrane domain (TM), and a cytoplasmic repeat (*cdc10/SWI6*) are indicated. Clones 13a, 24a, 28a and 31a were obtained from a library provided by J. Ahringer and J. Kimble; 13a and 24a were isolated after screening 3×10^6 plaques using the *Bgl*II fragment containing the predicted 3' end of the gene as a probe, 28a was found after screening 1×10^6 plaques using as probe the *Pst*I fragment including open reading frames (ORFs) A-C, and 31a was found after screening 2.5×10^6 plaques using as probe the *Eco*RI fragment including ORF Z. Clone 36a was obtained by screening 2×10^6 clones from a library provided by J. Goddard and M. Capecchi, and clones 51-1 and 62c were obtained by screening 7.5×10^5 clones from a library provided by S. Kim and R. Horvitz, with a fragment containing the predicted 3' end derived from the *Bgl*II fragment. Open arrows representing 51-1 and 62c indicate that these clones extend further 5' into ORF A based on restriction mapping. All three libraries were constructed using RNA from mixed-stage populations of wild-type (N2) worms and contain *Eco*RI linkers as cloning sites. **b**, The DNA sequence of a composite cDNA is shown in upper case and flanking genomic DNA is indicated in lower case, as are the ten base pairs forming the 5' or 3' ends of introns. The remaining number of base pairs within the introns are indicated. Also shown in upper case is part of a complete cDNA clone that maps 3' of *lin-12* and in the opposite orientation. The product deduced from this sequence is 40% identical with the P subunit of glutathione S-transferase from rat²³. A potential signal sequence and transmembrane domain are shaded; no other extensive hydrophobic regions are present in the sequence. Potential asparagine glycosylation sites²⁴ present in the predicted extracellular portion of the protein (see text) are circled; potential serine or threonine phosphorylation sites²⁵ in the intracellular portion are overlined; and potential poly(A)⁺ addition sites²⁶ are underlined in the 3' untranslated region of *lin-12*. The 3' ends of three cDNA clones are shown with arrow heads; although all lacked poly(A)⁺ tails, all end near a potential poly(A)⁺ addition site. We do not yet know if the 5' end of cDNA clone 31a corresponds to the 5' end of the primary transcript, although an (A + T)-rich region which may function as a 'TATA box' lies 37 base pairs upstream. General molecular methods used are described in ref. 27.

S P V C Q C K N G F I G K R C E K E C P I G F G G V R C D L R L E I G I C S R Q 465
 TCACCCGTATGTCACTGTAAACCGGATTATTGGAAAAACGATGTGAAAAGGAATGTCCAATCGGATTCGGAGGTGTCGGTGTGATCTTCGATTGGAAATGGAAATATGCTCAGACAA 1449

G G K C F N G G K C L S G F C V C P P D F T G N Q C E V N R K N G K S S L S E N 505
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L C L S D P C M N (N) A T C I D V D A H I G Y A C I C K Q G F E G D I C E R H K 544
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D L C L E N P C S N G G V C H Q H R E S F S C D C P P G F Y G N 576
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G C E Q E K M F R C L K S T C Q N G G V C I N E E E K G R K C E 608
 GGTGTGAACAGGAAAAATGTCCGCTGTCTCAAGAGgtgagatttt-26-aatatattcagCACTTGCCAAAACGGAGGAGTTGTATTATGAAGAAGAGAGGGAAGAAATGTGAA 1878

C S Y G F S G A R C E E K I (N) L T G F T E K D S L L R S V C E K 640
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R K C S E R A N D G N C D A D C N Y A A C K F D G G D C S G K R E P F S K C R Y 680
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G N M C A D F F A N G V C N Q A C N N E E C L Y D G M D C L P A V V R C P V K I 720
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R E H C A S R F A N G I C D P E C N T N G C G F D G G D C D (N) E T (N) A T I I T N 760
 CGAGAACATTGTGCTTCAAGATTGTGCAAAATGGAATCTGTGATCCAGAAATGAATACAAATGGATGTGGTTTTGATGGTGGTGTGATGATAATGAAACGAATGCCACAATTATCACAAAT 2334

I R I T V Q M D P K E F Q V T G G Q S L M E I S S A L R V T V R I Q R D E E G P 800
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L V F Q W N G E S E M D R V K M N E R Q L T E Q H V L S T S I S R K I K R S A T 840
 CTCGTGTTTCAATGGAATGGGGAATCTGAAATGGATCGAGTCAAAATGAATGAGAGACAGCTCACCGAGCAACATGTTCTTCCACATCAATTTCCAGAAAAATCAAGAGAGTGCACAG 2574

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 AATATTGGAGTTGTGCTCTATTTGGAAGTTCAAGAGAATTGTGATACAGAAAAATGCTCTACAAGGATGCTCAAAGTGTGTTGACTCAATATCTGCAAGGCTTCAAAGAAGGGAATC 2694

D S F G I P I S E A L V A E P R K S G (N) N T G F L S W N A L L L I G A G C L I V 920
 GATTCTTTTGGTATTCGAATCTCAGAAGCTCTTGTGCGAGAGCCAAGAAAAATCTGGAACAATACAGGATTCATTATCATGGAATGCTCTTCTTCAATTGGTGTGATGTCTGATTGTT 2814

M V V L M L G A L P G N R T R K R R M I N A S V W M P P M E N E E K N R K N H Q 960
 ATGGTTGTTCTGATGTTAGGAGCATTACCTGGAATCGGACAAGGAACGTCGAATGATCAACGCCCTCTGTCTGGATGCCACCTATGGAGAATGAGGAGAAGATCGGAAAAACCATCAA 2934

S I T S S Q H S L L E A S Y D G Y I K R Q R N E L Q H Y S L Y P N P Q G Y G N G 1000
 TCAATAACTTCAAGTACGATTCACCTTCTGGAAGCTTCTTATGATGGTTATATCAAAAGGCAAGAAATGAACCTCAACACTATTCCTTGTATCCAAATCCCTCAAGGATACGGGAAGGA 3054

N D F L G D F N H T N L Q I P T E P E P E S P I K L H T E A A G S Y A I T E P I 1040
 AATGATTTCCTTGGTGTATTCACCATACCAATCTTCAAATTCCAACTGAACCTGAACCTGAAAGGCCCATCAAACCTACACACAGAAGCTGAGGAAGCTATGCAATTTACTGAGCCTATT 3174

T R E S V N I I D P R H N R T V L H W I A S N S S A E K S E D L I V H E A K E C 1080
 ACTAGGGAATCCGTGAATATTATTGATCCAAGGCACAAATAGAAGGTTTTCGATTGGATTGCATCGAATTCCTCAGCCGAAAAGTCAGAAGATTGATTGTACATGAAGCTAAAGAATGT 3294

I A A G A D V N A M D C D E N T P L M L A V L A R R R R L V A Y L M K A G A D P 1120
 ATTGCGCAGAGCCGATGTGAATGCTATGGATTGCGACGAGAACACCCCATGTGCTGGCTGTTCTGCAAGAAGACGACGCTTGTGGCTTATCTGATGAAAGCTGGAGCCGATCCA 3414

T I Y N K S E R S A L H Q A A A N R D F G M M V Y M L N S T K L K G D I E E L D 1160
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R N G M T A L M I V A H N E G R D Q V A S A K L L V E K G A K V D Y D G A A R K 1200
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G K T P I M L A A Q E G R I E V V M Y L I Q Q G A S V E A V D A T 1273
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D H T A R Q L A Q A N N H H N I V D I F D R C R P E R E Y S M D L H I Q H T 1311
 ttccagGATCATACGGCTCGTCAACTCGCTCAAGCAATAACCATCACAATATTGTTGATATTTTGTAGATGTGCTCCAGAACGAGAATATTCAATGGATCTTCACATCCAACATACC 3987

H Q P Q P S R K V T R A P K K K Q T S R S K K E S A S N S R D S T H L T P P P S D 1351
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G S T S T P S P Q H F M N T T H T P T S L N Y L S P E Y Q T E A G S S E A 1389
 GGATCAACGTCAACACCGTCAACCACGATTTTATGAATACACTCACTACACCGACGAGTTTGAACATTTTGAAGCCAGAATATCAAACTGAAGCTGGAAGCTCAGAAGCGgttagt 4221

F Q P Q C G A F G N G E M W Y T R A S T S Y T Q M Q N E P M T R Y 1422
 tttta- 367 -ttactttcagTTCCAACCACAATGTGGAGCATTGGGAAATGGCGAAATGTGGTATACAAGAGCTTCAACATCGTACACCCAAATGCAGAAATGAACCAATGACCGGATAC 4320

S E P A H Y F * 1429
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TTTTGTTGCATTCTTGATTGTATATGGGCTTACGGGTGAAATGTTTGTGTAATGTACATTAATAAAAAATTAGTGAATAATtttccgagtattggtccccaacgaacgactgaaacgat 4524
 36a ▲ 51-1▲ 62c 3'AAAAAA

gtatgggaagcagagattcattggaagctttaaggaagacataaattatatcaaaactggaatttttgatgattgcaaaatgagaaaaattgaattagcttattgttttccatttccat
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 * Q K G N G N

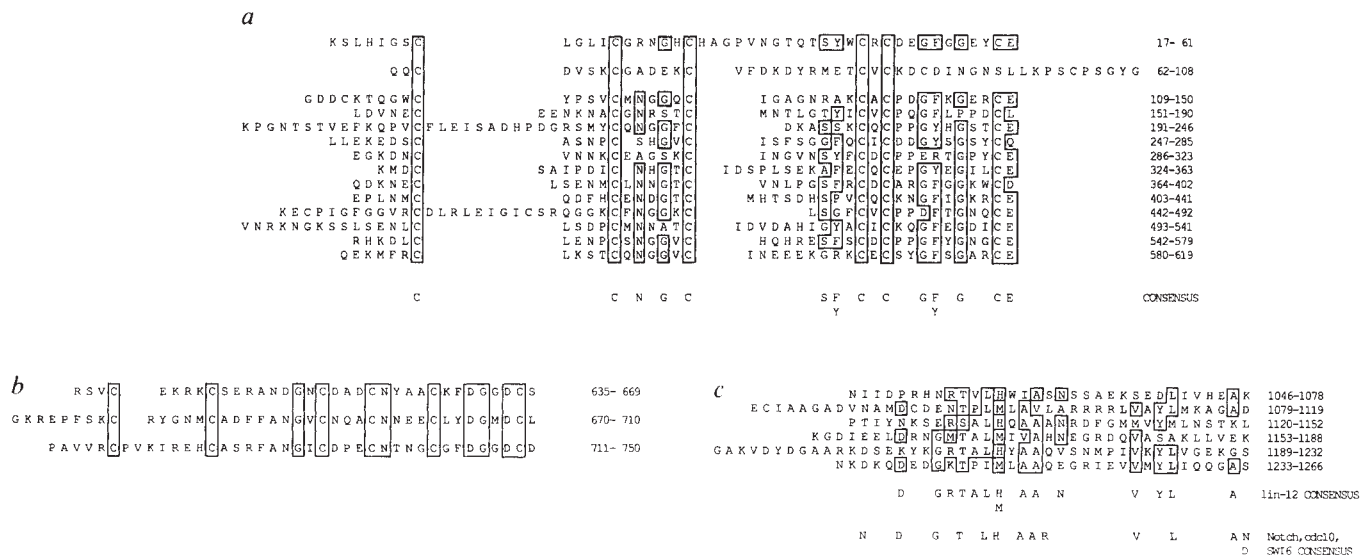


Fig. 2 Repeated motifs within the amino acid sequence deduced for *lin-12*. **a**, The 13 EGF-like (EGFL) repeats are aligned with respect to cysteine residues. The consensus sequence is based on the presence of a residue at least 7 times: identical amino acids are boxed, as are the related amino acids phenylalanine and tyrosine. Shown between the first and second repeats is a sequence formed by a splice of ORF T to Y that differs in its carboxyl half in every position from the consensus derived for the EGFL repeats in *lin-12* and those in other proteins. The second EGFL starts with ORF Z. **b**, Three cysteine-rich repeats (LNR1-3, for *lin-12*/Notch repeat) that lie between the final EGFL repeat and the potential transmembrane domain are aligned to show conserved residues. **c**, An alignment of six repeats of a motif within the cytoplasmic portion reveals a consensus sequence similar to a consensus that can be derived from the alignment of Breeden and Nasmyth⁹ for a motif repeated in the cytoplasmic domain of the *Drosophila Notch* gene product, twice in the *Schizosaccharomyces pombe cdc10* product, and twice in the *SW16* product from *Saccharomyces cerevisiae*. The *lin-12* consensus is based on a residue occurring at least three times.

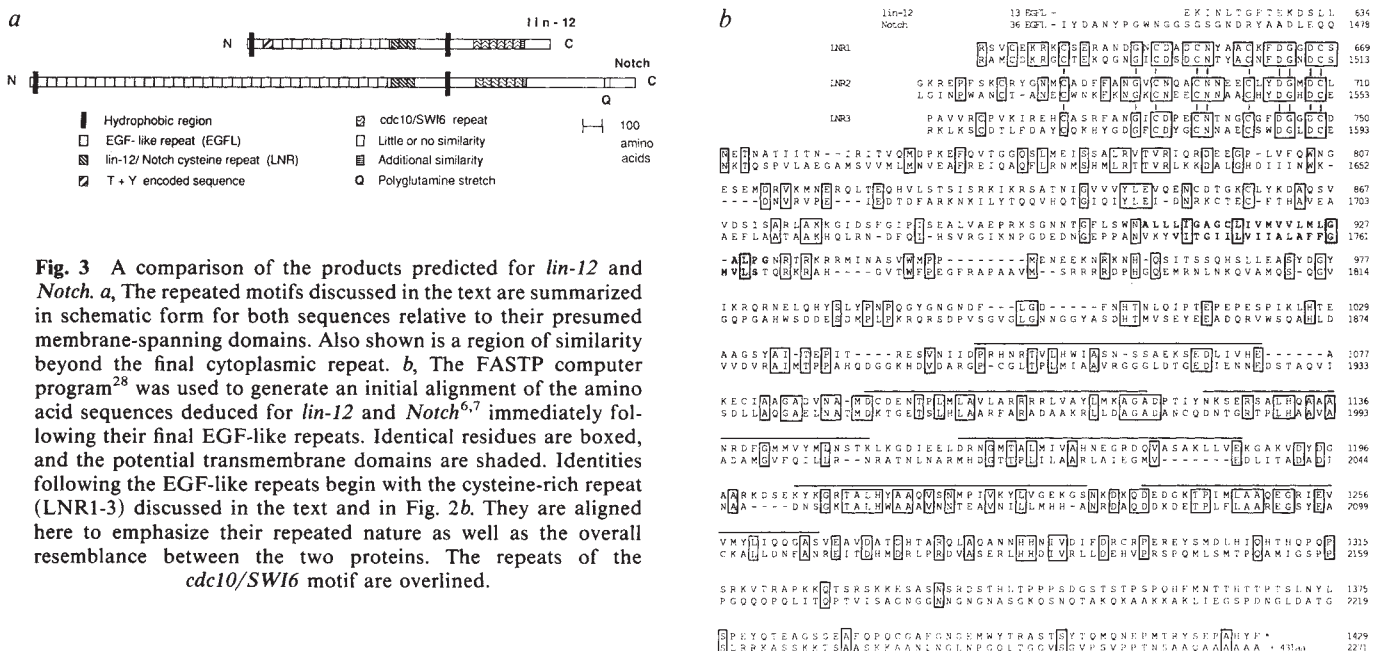


Fig. 3 A comparison of the products predicted for *lin-12* and *Notch*. **a**, The repeated motifs discussed in the text are summarized in schematic form for both sequences relative to their presumed membrane-spanning domains. Also shown is a region of similarity beyond the final cytoplasmic repeat. **b**, The FASTP computer program²⁸ was used to generate an initial alignment of the amino acid sequences deduced for *lin-12* and *Notch*^{6,7} immediately following their final EGF-like repeats. Identical residues are boxed, and the potential transmembrane domains are shaded. Identities following the EGF-like repeats begin with the cysteine-rich repeat (LNR1-3) discussed in the text and in Fig. 2b. They are aligned here to emphasize their repeated nature as well as the overall resemblance between the two proteins. The repeats of the *cdc10*/SW16 motif are overlined.

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Lateral inhibition during vulval induction in *Caenorhabditis elegans*

Paul W. Sternberg

Division of Biology 156-29, California Institute of Technology, Pasadena, California 91125, USA

During *Caenorhabditis elegans* vulval induction the anchor cell of the gonad specifies a spatial pattern of three cell types among a set of six multipotent epidermal cells, the vulval precursor cells (VPCs)¹⁻⁴. Previous studies suggested that the anchor cell produces a graded inductive signal which can directly stimulate VPCs away from a ground state (type 3) to become type 1 or type 2 depending on their distance from the anchor cell⁴. Here, we investigate the interactions among VPCs in a mutant, *lin-15*, in which VPC fates are rendered partially independent of the inductive signal^{5,6}, and show that type 1 cells actively inhibit adjacent cells from also becoming type 1 cells. The fate of each VPC therefore depends on the combined action of two intercellular signals: a graded inductive signal from the anchor cell, and a lateral inhibitory signal from at least some of its neighbours. Pattern formation among the VPCs in *lin-15* mutant is analogous to the establishment of the pattern of neuroblasts and dermatoblasts during early insect neurogenesis^{7,8}, suggesting that the similarities in inferred molecular structure of the *lin-12* and *Notch* gene products, which are involved in these two instances of pattern formation⁹⁻¹², might extend to similarities in function.

The vulval precursor cells (VPCs) are a set of six homologous epidermal cells (P3.p, P4.p, P5.p, P6.p, P7.p and P8.p) formed during the first post-embryonic larval stage of *C. elegans* hermaphrodites¹. Although each VPC is multipotent^{2,4}, with the potential to become a type 1, 2 or 3 cell, the spatial pattern of cell types is invariant in an intact animal¹ (Fig. 1a). Each VPC type is deduced from its subsequent developmental fate—the production of a particular set of progeny cells (Table 1 animals A). Type 1 and 2 VPCs generate distinct sets of progeny that differentiate as vulval tissue. A type 3 VPC generates two non-vulval epidermal cells that fuse with a large epidermal syncytium, *hyp7* (ref. 13). The ground state of VPC is to become type 3, and the anchor cell is necessary to stimulate VPCs away from the ground state: in the absence of the anchor cell of the gonad, all VPCs become type 3 (Table 1 animal B; Fig. 1b; ref. 3); moreover, in the absence of neighbouring VPCs as well as the anchor cell, P7.p becomes type 3, indicating that the anchor cell is not necessary only to overcome an inhibitory signal among the VPCs (Table 1, animals C; Fig. 1c; ref. 4). Previous studies indicated that the signal from the anchor cell is graded⁴. Specifically, in the absence of other VPCs, the fate of a single VPC is correlated with its position with respect to the anchor cell: when close to the anchor cell a VPC becomes type 1, when distant, type 3, and when at an intermediate position, type 2. During normal development, P6.p is invariably the closest cell to the anchor cell and becomes type 1. After destruction of P6.p, a more distal VPC can replace it, moving into the place of the destroyed cell and becoming type 1 (ref. 2). Such regulation implies that P6.p normally prevents a more distal VPC from becoming type 1, either by passively blocking its access to the inductive signal, or by actively inhibiting it. Because the response by the VPCs to the anchor cell prevents study of interactions among the VPCs, I have examined the interactions of VPCs in a mutant in which the VPCs are rendered partially independent of the inductive signal.

The *lin-15*(n309) mutation causes hermaphrodites to have two or three pseudovulvae in addition to a functional vulva, the 'multivulva' phenotype⁵. In *lin-15* hermaphrodites, all six VPCs are type 1 or type 2, and none are type 3 (ref. 6; Table 1, animals 1D). In the absence of the anchor cell, VPCs are still type 1 or

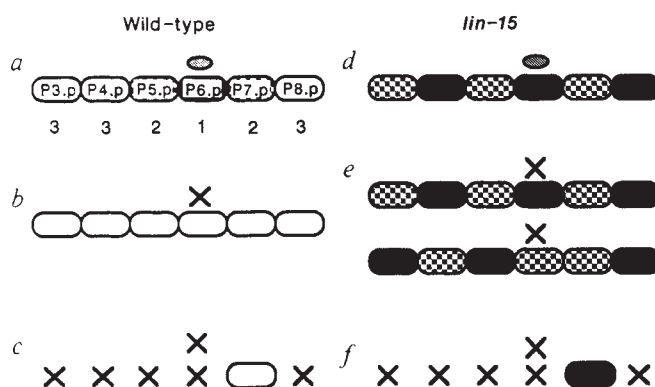


Fig. 1 Schematic illustration of key experimental observations and controls condensed from the data in Table 1. X, cell destroyed by irradiation. White oval, type 3 cell; chequered oval, type 2 cell; black oval, type 1 cell; stippled oval, anchor cell. Each part (a-f) corresponds to the similarly lettered animals in Table 1. In d and e, the patterns shown are typical; see Table 1 for range of variation.

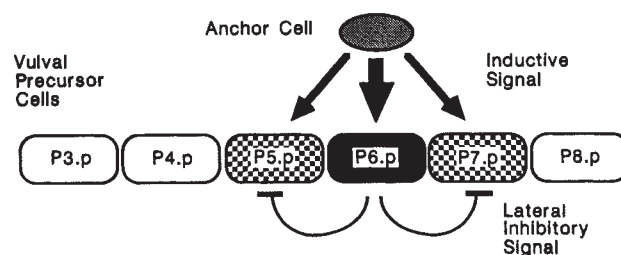


Fig. 2 Model for pattern formation during vulval induction. As described in the text, six initially equivalent cells, the VPCs, are subject to a graded inductive signal from the anchor cell, and a lateral inhibitory signal from at least some of their neighbours. The anchor cell stimulates P5.p, P6.p and P7.p away from the ground state, type 3 (white oval). These three cells compete for the type 1 fate (black oval) by producing inhibitory signal (or by becoming refractory to it). P6.p wins because it receives the inductive signal at a higher level or at an earlier time than do P5.p and P7.p and is thus able to inhibit them before it becomes inhibited. According to this model, a VPC can become type 2 (chequered oval) either by receiving an intermediate level of inductive signal, for example P8.p in the absence of the other five VPCs⁴, or by receiving a high level of inductive signal from the anchor cell and receiving inhibitory signal from P6.p, e.g., P7.p in an intact animal. The possibility that either only the inductive signal or only the LIS acts on P5.p and P7.p during normal development cannot be excluded. It is possible that type 2 cells inhibit more distal neighbours (P4.p and P8.p) from also becoming type 2, either by the LIS or another inhibitory signal.

type 2 (Table 1, animals E; Fig. 1e), although the patterns of fates are slightly different. In *lin-15* animals, both with and without an anchor cell, there is a striking alternating pattern of type 1 and type 2 fates. In the 14 *lin-15* animals described in Table 1 (animals D and E) adjacent cells were type 1 in only one of 70 pairs of cells, whereas adjacent cells were type 2 in nine of 70 pairs. These observations suggested that a type 1 cell prevents its neighbours from also being type 1. To test this hypothesis, I destroyed, by irradiation from a laser microbeam, the precursors to the anchor cell and five of the six VPCs, leaving P7.p intact. In all eight such *lin-15* hermaphrodites, P7.p was type 1 (Table 1, animals F). By contrast, in seven of eight animals lacking the anchor cell but with all six VPCs intact, P7.p was type 2 (Table 1, animals E). Therefore, the ground state of a VPC in a *lin-15* animal is to be type 1. If a neighbouring cell is type 1, a VPC will become type 2, suggesting that the type 1 cell is inhibiting its neighbours from also becoming type 1. I

Table 1 Fates of VPCs in experimental animals

Genotype	AC	P3.p	P4.p	P5.p	P6.p	P7.p	P8.p
(A) Wild-type	+	S S	S S	LLTN	TTTT	NTLL	S S
(B) Wild-type	—	S S	S S	S S	S S	S S	S S
(C) Wild-type	—	—	—	—	—	S S	—
	—	—	—	—	—	S S	—
	—	—	—	—	—	S S	—
	—	—	—	—	—	S S	—
	—	—	—	—	—	S S	—
	—	—	—	—	—	S S	—
	—	—	—	—	—	S S	—
(D) <i>lin-15</i>	+	LLTT	LOTO	LLLN	TTTO	NLLL	LOOO
	+	LOLL	OTTT	LLLT	TTTT	TLLL	TTTL
	+	LT TT	LLTN	LLON	TTTT	NTLL	LT TL
	+	TOOT	LLON	LLTT	TTTT	NLLL	OTTT
	+	OLLO	TTTT	LLTT	TTTT	NTLL	TTLL
	+	LLTN	TTTL	LLON	TTTT	NOLL	OTLL
(E) <i>lin-15</i>	—	OTTT	LLON	OTTT	LLTT	NTLL	OTTT
	—	LT TL	NOLL	OTTT	NTLL	TTLL	DOTL
	—	TLLL	LLLN	OOTO	LLTT	LLLT	OOTO
	—	LLLN	OTTT	LLLN	TOTT	NOLL	OTOT
	—	OLTN	OTTT	LLLN	LT TT	NTLL	OTTT
	—	LLLO	OTTO	LLOT	TTTT	NOLL	LT TT
	—	LTOL	LLOO	LLLT	OTTO	NTLL	OTTT
	—	TTTT	DLON	TOOL	LLTN	TOTO	DDDD
(F) <i>lin-15</i>	—	—	—	—	—	LT TL	—
	—	—	—	—	—	LOOL	—
	—	—	—	—	—	LLOL	—
	—	—	—	—	—	OOTL	—
	—	—	—	—	—	OTOO	—
	—	—	—	—	—	LOTO	—
	—	—	—	—	—	LOOO	—
	—	—	—	—	—	DDDD	—
(G) <i>lin-15</i>	+	—	—	—	—	TTTT	—
	+	—	—	—	—	LOOL	—
	+	—	—	—	—	DDDD	—
(H) <i>lin-15</i>	—	—	—	—	—	LOLN	LTOL
	—	—	—	—	—	LLOO	LOTL
	—	—	—	—	—	OTLL	TOTL
	—	—	—	—	—	TTLL	LLLL
	—	—	—	—	—	OTTO	OLLL
	—	—	—	—	—	LT TL	NOLL
	—	—	—	—	—	LLOO	LLOT
	—	—	—	—	—	TTDL	TTOO
	—	—	—	—	—	LLOL	LT TL

refer to this inhibitory signal as the 'lateral inhibitory signal' (LIS).

Although these experiments have concentrated on P7.p, the alternating pattern in *lin-15* animals (Table 1, animals D) suggests that the LIS is acting among all six VPCs. Also, in 22 additional experimental animals (data not shown), I examined the patterns of cell types in animals in which one, two or three VPCs, as well as the anchor cell, were destroyed. In all cases the results were consistent with the hypothesis that the LIS can be produced by all six VPCs. The alternating pattern of type 1

and 2 cell fates is a consequence neither of the particular *lin-15* mutation used nor of the mutant *lin-15* gene *per se*: other multivulva mutants, *lin-15(n765)*, *lin-13*, and CB1322 (*lin-8; lin-9*), also have alternating patterns of type 1 and type 2 VPCs (ref. 6 and unpublished data).

To determine whether the LIS works at a distance, I examined *lin-15* animals in which the anchor cell and four of the six VPCs were destroyed, leaving two VPCs intact. In animals with only P7.p and P8.p intact, the spacing of these cells was variable because the cells sometimes moved apart. Three patterns of cell

Table 1 continued

Genotype	AC	P3.p	P4.p	P5.p	P6.p	P7.p	P8.p
(I) <i>lin-15</i>	—	OTTT	—	—	—	LTTT	—
	—	TTTT	—	—	—	LOOL	—
	—	TLTL	—	—	—	OTLL	—
	—	TTTT	—	—	—	TTTT	—
	—	TTTL	—	—	—	LOLL	—
	—	LLLO	—	—	—	LOLO	—
	—	LOTT	—	—	—	LLLL	—
(J) <i>lin-15</i>	+	—	—	—	—	LTTO	TLLL
	+	—	—	—	—	OTTT	TLLL
	+	—	—	—	—	OOTO	TLLL
	+	—	—	—	—	LOTT	TLLL
	+	—	—	—	—	LTTO	TTLL
	+	—	—	—	—	OTTT	TLLL
	+	—	—	—	—	TOOT	TTLL
	+	—	—	—	—	OTTT	NOLL
	+	—	—	—	—	LOTT	TLLL
	+	—	—	—	—	TTTO	TLLL
	+	—	—	—	—	OTTO	LTOO
	+	—	—	—	—	TTTT	TLLL
	+	—	—	—	—	TTTO	LLLL
	+	—	—	—	—	LLTT	TOLL

The fate of each VPC is inferred, as described below, from the pattern of cell division and progeny cell types it produces. Each line in the table represents one experimental animal. A box indicates a type 1 lineage. With the exception of animals A, B and C, an unboxed lineage is type 2; in A, B and C [S S] represents a type 3 lineage, and [LLTN] or [NTLL] represent type 2 lineages. Fates of remaining VPC cells in intact wild-type animals^{1,4} (A), wild-type animals lacking the anchor cell^{2,3} (B), wild-type animals lacking an anchor cell and all VPCs but P7.p (C), intact *lin-15* animals (D), *lin-15* animals lacking the anchor cell (E), *lin-15* animals lacking the anchor cell and all VPCs but P7.p (F), *lin-15* animals lacking all VPCs but P7.p (G), *lin-15* animals lacking the anchor cell and all VPCs but P7.p and P8.p (H), *lin-15* animals lacking the anchor cell and all VPCs but P3.p and P8.p (J), and *lin-15* animals lacking all VPCs but P7.p and P8.p (J). AC, anchor cell; +, anchor cell present; —, anchor cell absent because its ancestors were destroyed by irradiation with a laser microbeam. A dash indicates that a relevant VPC was destroyed. Criteria for type 1, type 2 and type 3 sublineages have been established by Sternberg and Horvitz⁴. Each symbol represents the fate of a VPC daughter (Pn.pa or Pn.pp, for $n=1-8$) or grand-daughter (Pn.paa, Pn.pap, Pn.ppa, Pn.ppp): L, divides with a longitudinal axis; T, divides with a transverse axis; N, does not divide; S, joins the epidermal syncytium. In addition, two other symbols are used to indicate abnormal or unobserved division axes: O, divides with an oblique axis intermediate between T and L; D, divided but division axis not observed. Boldfacing indicates adherence to the ventral cuticle instead of invagination. The hallmark of a type 1 lineage is the absence of adherence to the ventral cuticle, and the lack of an N cell. The canonical type 1 lineage is [TTTT]; oblique or longitudinal division axes are acceptable if none of the cells adhere to the ventral cuticle. The hallmark of a type 2 lineage is the presence of two adherent cells that divide longitudinally. The canonical type 2 lineages are [LLTN] and [NTLL] or [LLTT] and [TTLL]. Oblique (O) division axes are acceptable as part of a type 2 lineage if either Pn.paa and Pn.pap, or Pp.ppa and Pn.ppp adhere to the ventral cuticle. The hallmark of a type 3 lineage is the generation of two S daughters. The canonical type 3 lineage is [S S]; in wild-type, P3.p sometimes joins the large epidermal syncytium without dividing. Differences in the details of the cell lineages (for example, [TTTT] compared to [LTTT]) presumably reflect randomness arising from either of two sources: cell interactions occurring among the grand-daughters of the VPCs, or constraints on the axis of cell divisions. For example, in a wild-type animal in which all but one VPC have been destroyed, a VPC at the anchor cell undergoing a type 1 lineage can generate an [LTTT] lineage⁴, the axes of the outer cells no longer constrained by neighbouring cells. Isolated type 2 lineages in wild-type are often [LLTT]. The slightly abnormal type 1 lineages are also seen if the anchor cell is present (G) indicating that the abnormalities are a consequence of a cell being isolated, or conceivably other effects of *lin-15*, as opposed to some novel VPC type. In most cases in which both of two remaining VPCs were type 1, the VPCs were separated; however, in one case (last line of Table) both were clearly touching and next to the anchor cell. Strains used were *lin-15(n309)* (ref. 5) and the wild-type strain N2 (ref. 18). General methods have been described by Brenner¹⁸. Laser killing of cells was performed by the method of Sulston and White² using a laser microbeam system essentially identical to that described by Avery and Horvitz¹⁹. The beam from a coumarin dye (7-amino-4-methylcoumarin [Sigma] at 5 mM in ethanol) laser was focused through a $\times 10$ eyepiece and passed without neutral density filter into the epicondenser of a Zeiss Standard 16 microscope equipped with Nomarski DIC optics. Animals were picked into 2 μ l of S-Basal¹ placed on a pad of 5% agar in water containing 10 μ M sodium azide as anaesthetic¹⁹. This preparation was covered with an 18 $\times$ 18 mm cover glass. Killing was checked 4–8 hours after operation. Cell lineages were followed as described by Sulston and Horvitz¹. Cells were destroyed between the middle of the L1 stage (9 h post-hatching) and the beginning of the L2 stage (17 h post-hatching); no differences were observed as a function of the time of operation. Anchor cells were removed by killing Z1 and Z4, or Z1.p and Z4.a. In control animals, when Z2 and Z3 were ablated, or Z4 was ablated, no effect on vulval development was observed (data not shown).

fates were observed, 1–2, 2–1 and 1–1 (Table 1, animals H); in those animals in which P7.p and P8.p both became type 1, P7.p and P8.p had moved apart. In six of seven animals with only P3.p and P7.p intact, these two cells remained apart, and a 1–1 pattern of fates was observed (Table 1, animals I). In the seventh animal, P3.p moved posteriorly until it was adjacent to P7.p and the pattern of fates was 2–1. These data are consistent with the hypothesis that the LIS only works over a short distance, possibly requiring direct contact between the cells.

The alternating pattern of VPC fates observed in *lin-15* animals is analogous to other examples of pattern formation in which neighbouring multipotent cells are inhibited from becoming a 'primary' cell type, such as bristle precursors in *Rhodnius*¹⁴,

or heterocysts in *Anabaena*¹⁵. Such random but accurately-spaced patterns might arise because cross-inhibitory interactions between cells result in an initial state that is metastable, and random fluctuations push the system to any of a set of stable states¹⁵. In *lin-15* mutants each of the six VPCs might be poised between the alternative pathways of differentiating as type 1 or type 2. The observed patterns would arise because the VPCs compete for the type 1 fate, and the field of cells eventually settles into one of a few stable patterns of cell types, for example, 2–1–2–1–2–1 or 1–2–2–1–2–1. During grasshopper early neurogenesis, presumptive neuroblasts inhibit neighbouring dermatoblasts from also becoming neuroblasts⁷; these cell interactions are also thought to occur during neurogenesis of *Drosophila*

*melanogaster*⁸. The analogy between the cell-cell interactions underlying nematode vulval development and insect neurogenesis might extend to related molecular mechanisms because similar gene products have been implicated in the specification of the 'inhibited' or 'secondary' fate. Specifically, in *C. elegans* the *lin-12* product is necessary for the type 2 fate¹⁶, and in *D. melanogaster* the *Notch* product is necessary for the dermatoblast fate (reviewed in ref. 17). These genes, which have extensive sequence similarity⁹⁻¹², have been proposed to mediate cell-cell contact signalling events⁹⁻¹²; such a role would be consistent with our observation that the LIS is more effective over a short distance.

I have described how VPCs in *lin-15* mutants are susceptible to an inhibitory signal. Two additional observations suggest that the VPCs in a *lin-15* mutant are also responsive to the inductive signal from the anchor cell. In the presence of the anchor cell and P8.p, P7.p became type 1 in all fourteen animals examined (Table 1, animals J). By contrast, in the absence of an anchor cell, P7.p became type 2 in four of nine cases (Table 1, animals H). The anchor cell thus biases P7.p to become type 1, presumably due to the inductive signal. Moreover, in *lin-15* animals with intact VPCs, P6.p became type 1 in all six cases with the anchor cell, but became type 2 in four of eight cases without the anchor cell (Table 1, animals D and E). Therefore, although a *lin-15* mutation allows VPCs to become type 1 or type 2 in

the absence of inductive signal from the anchor cell, the cells are still responsive to the inductive signal.

I propose that the precise pattern of VPC fates results from the combined action of two intercellular signals (Fig. 2): a graded inductive signal from the anchor cell, and a signal from type 1 cells to type 2 cells. In this view, the anchor cell induces three cells away from the ground state, thereby allowing them to become type 1 or 2. The three induced VPCs compete for the type 1 fate; P6.p always wins because it is closer to the anchor cell and hence receives inductive signal sooner or at a higher level than P5.p and P7.p, which become type 2. Although the signal from type 1 to type 2 cells seems to be inhibitory, preventing a cell that receives it from becoming type 1, it is possible that the LIS stimulates a VPC to become type 2, thereby precluding it from becoming type 1. For example, differentiation as type 1 and type 2 might be the result of exclusive cell states, and thus to promote type 2 is to inhibit type 1. If so, *lin-12*, necessary for the specification of types 2 cells¹⁶, might act in the LIS pathway.

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Insulin stimulation of glucose uptake can be mediated by diacylglycerol in adipocytes

Peter Strålfors

Department of Physiological Chemistry, University of Lund,
PO Box 94, S-22100 Lund, Sweden

An early effect of insulin in adipocytes is to stimulate glucose uptake. The increased uptake appears to be due to mobilization of glucose transporters from an intracellular location to the plasma membrane^{1,2} and to enhanced intrinsic activity of the transporters³⁻⁷. Little is known about the insulin-generated signals causing these changes. Phorbol esters have been shown to mimic the insulin effect^{8,9}, but phosphorylation of the transporter does not seem to be involved^{10,11}. A phospho-oligosaccharide was recently shown to mimic the effects of insulin on protein phosphorylation, suggesting that it could be a mediator for some intracellular metabolic effects of the hormone¹², but it did not affect glucose uptake¹³. A diacylglycerol is produced in the plasma membrane in conjunction with the generation of the phospho-oligosaccharide¹⁴. Here I show that added 1,2-diacylglycerols potently increase glucose transporter-mediated uptake of glucose in rat adipocytes, but without activation of protein kinase C.

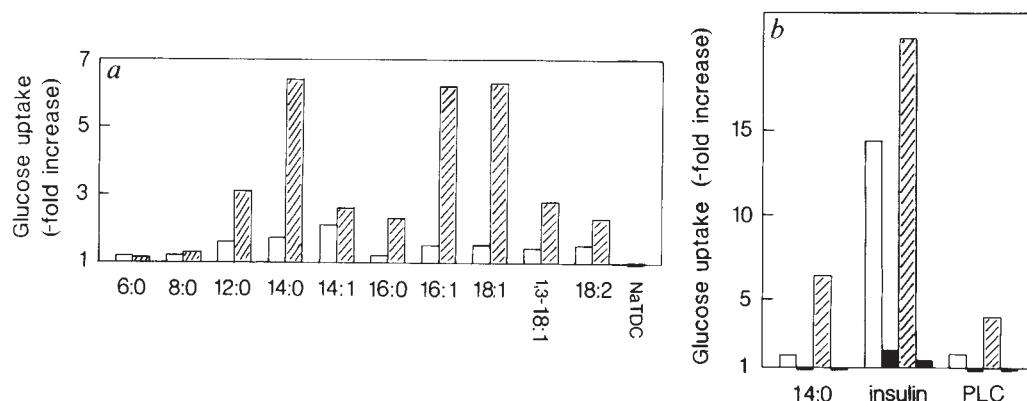
1,2-Diacylglycerols and analogues were presented to cells as an emulsion. To enhance the transfer of diacylglycerol from the emulsion droplets to the plasma membrane of the cells, the diacylglycerol was added with a bile salt¹⁵. Sodium taurodeoxycholate (NaTDC) at 0.4 mM enhanced the transfer of 1,2-di[³H]octadecenoylglycerol at 20 μ M to the cells

(12,100 $\pm$ 1,600 c.p.m. without, and 23,400 $\pm$ 1,000 c.p.m. with NaTDC (mean $\pm$ s.e., $n = 4$) was associated with the floated and washed cells). This corresponds to approximately 1% of total plasma membrane lipid molecules (assuming 5×10^6 molecules of lipid per μ m² of plasma membrane), but the exact amount incorporated into the cells is difficult to assess because the diacylglycerol emulsion tends to float with the cells during washing. NaTDC also enhanced the stimulatory effect of diacylglycerols on glucose uptake, but had no effect by itself (Fig. 1a). Interestingly, NaTDC amplified the stimulatory effect of insulin (Fig. 1b). The optimal diacylglycerols were 1,2-ditetradecanoylglycerol, 1,2-dihexadecenoylglycerol and 1,2-dioctadecenoylglycerol (Fig. 1a). The transfer efficiency of the different diacylglycerols to the cells was not determined, so the effect of a particular diacylglycerol could be underestimated. 1,3-Dioctadecenoylglycerol was considerably less effective in inducing glucose uptake than was the positional 1,2-isomer (Fig. 1a). Diacylglycerol analogues with ether linkages in the 1-(1-octadecenyl-2-octadecenoylglycerol) or 2-(1-octadecenyl-2-octadecenoylglycerol) position or in both positions (1,2-dioctadecenoylglycerol) were found to be ineffective (data not shown).

Phospholipase C added exogenously to the cells stimulated glucose uptake (Fig. 2) in a dose-dependent manner, but at high doses ultimately destroyed the cells. The phospholipase C effect was enhanced by the presence of NaTDC. The stimulation of glucose uptake by insulin, diacylglycerol or phospholipase C, in the presence or absence of NaTDC, was effectively blocked by the inhibitor of the glucose transporter cytochalasin B (Fig. 1b).

In the presence of NaTDC the stimulation by 1,2-ditetradecanoylglycerol increased with increasing concentration up to about 100 μ M, with EC₅₀ of approximately 20 μ M (Fig. 3). The time course of diacylglycerol stimulation of glucose-transport rate (measured by 3-O-methyl-D-glucose uptake,

Fig. 1 Effect of different 1,2-diacylglycerols and analogues on D-glucose uptake. *a*, Diacylglycerols (100 μ M) were incubated with cells. The notation for 1,2-diacylglycerols, 14:0 and so on, denotes number of carbons in the acyl chain; number of *cis*-double bonds in the acyl chain; 1,3-18:1, 1,3-dioctadecenoylglycerol; without (open bars) or with (hatched bars) 2 mM NaTDC. *b*, 1,2-ditetradecanoylglycerol (100 μ M), insulin (INS) (1 nM) or phospholipase C (PLC) (3 ng ml^{-1}) was incubated with cells in the absence (open bars) or presence (hatched bars) of 2 mM NaTDC. Incubations were in the absence or presence (filled bars) of 5 μ g ml^{-1} of cytochalasin B (Sigma).



Methods. Adipocytes were isolated from the epididymal fat pads of 130–150 g Sprague-Dawley rats (Alab, Sweden) which were starved overnight and killed by cervical dislocation. Cells (final concentration 15–20 μ l packed-cell volume per ml) were incubated in Krebs-Ringer buffer (0.13 M NaCl, 5 mM KCl, 2.5 mM CaCl_2 , 1.2 mM MgSO_4 , 1.2 mM KH_2PO_4) containing 20 mM HEPES, pH 7.40, 3.5% (w/v) defatted bovine serum albumin, 100 nM phenylisopropyladenosine. 0.5 U ml^{-1} adenosine deaminase and 0.5 mM D-glucose, at 37 °C on a shaking waterbath¹². After 1 h preincubation, incubations were started by adding 350 μ l cells to 7-ml scintillation vials (Packard) containing 50 μ l diacylglycerol or additives as indicated, plus 0.3 nCi of D-[3- ^3H]glucose (Amersham) and incubated for a further 60 min. Reactions were terminated by adding 3 ml scintillation cocktail (0.3 g POPPOP, 5 g PPO in 1 l of toluene) and vortexing to extract lipids into the organic scintillation phase²⁶. In the presence of the low glucose concentration used here, glucose uptake is rate-limiting in the conversion of glucose into lipids²⁷. Results are presented as the increase in toluene-extractable lipids compared with controls in the absence of additives. It was verified that the initial rate of 3-O-methyl-D-[3- ^3H]glucose (Amersham) (ref. 3) uptake was increased by 1,2-ditetradecanoylglycerol (not shown). Diacylglycerol emulsions were prepared by injecting a solution of the lipid in ethanol into medium with or without NaTDC. Final concentration of ethanol in cell incubations was 0.5% (v/v); this concentration of ethanol had no effect on glucose uptake. All experiments have been performed at least twice using different preparations of cells with similar results. 1,2-Dihexadecanoylglycerol was obtained from Larodan (Malmö, Sweden). 1-Octadecenyl-2-octadecenoylglycerol was prepared by pancreatic lipase hydrolysis of 1-octadecenyl-2,3-dioctadecenoylglycerol. All other diacylglycerols and their analogues were synthesized as in refs 28 and 29. All lipids were >99% pure judged by thin layer chromatography. Diacylglycerols were racemic mixtures of *sn*-1,2 and *sn*-2,3 enantiomers.

ref. 3) was similar to the time course of diacylglycerol (1,2-di[3- ^3H]octadecenoylglycerol) transfer to the cells, with half-maximal effects after ~20 min. In cells maximally stimulated by insulin (1 nM) plus NaTDC, the inclusion of 1,2-ditetradecanoylglycerol gave no further stimulation. In cells stimulated by insulin alone, the addition of 1,2-ditetradecanoylglycerol had an additive effect, but total stimulation did not exceed the maximal activity obtained in the presence of 1 nM insulin plus NaTDC (not shown).

1,2-Dioctanoylglycerol (an activator of protein kinase C in intact cells), which had only a very small stimulatory effect on glucose uptake (Fig. 1a), increased the phosphorylation of a protein with relative molecular mass (M_r) 40,000 (40K) in adipocytes prelabelled with [^{32}P]phosphate (Fig. 4). The same 40K protein was also phosphorylated in response to the protein kinase C activator 12-O-tetradecanoylphorbol-13-acetate TPA (Fig. 4). On the other hand, 1,2-ditetradecanoylglycerol did not produce any detectable changes in protein phosphorylation (Fig. 4).

These and other findings^{12,14,16–18} suggest a unifying hypothesis for the short-term mechanism of action of insulin: insulin activates a phospholipase C (refs 16–18) to produce a water-soluble phospho-oligosaccharide¹⁶ which may control the state of phosphorylation of insulin target enzymes¹². Diacylglycerol is also produced¹⁴ and may mediate rapid membrane events of the hormone. This scheme with dual second messengers is akin to the receptor-induced activation of phospholipase C acting on phosphoinositides to produce inositol-1,4,5-trisphosphate and diacylglycerol¹⁹. But protein kinase C activation is not involved in controlling glucose uptake, because the diacylglycerols that affected glucose uptake did not activate protein kinase C and vice versa. The added long-chain diacylglycerols did not affect protein kinase C activity, probably because of poor penetration to the inner plasma membrane leaflet²⁰. This is a potential way to distinguish between the diacylglycerols produced during phosphoinositide hydrolysis because these are produced in the inner leaflet of the plasma membrane and insulin

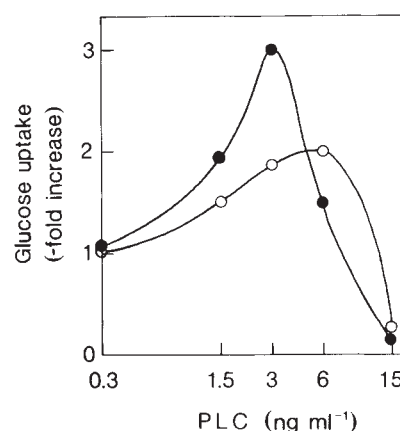


Fig. 2 Exogenous phospholipase C (PLC) stimulation of glucose uptake. Cells were incubated as described in the legend to Fig. 1. Phospholipase C at the indicated concentrations was incubated with cells in the absence (open circles) or presence (filled circles) of 2 mM NaTDC. Phospholipase C from *B. cereus* (type III, Sigma) was further purified by gel filtration on Superose 12 (Pharmacia) and was free from any phosphoinositide-specific phospholipase C.

hydrolysis of the phosphatidyl phospho-oligosaccharide occurs in the outer leaflet^{21,22}.

Both exogenous and endogenously produced 1,2-diacylglycerols enhanced glucose uptake, with an optimal effect for 1,2-ditetradecanoylglycerol. This diacylglycerol was produced from phosphatidyl phospho-oligosaccharide in response to insulin in a myocyte cell line¹⁴. In a hepatoma cell line the insulin-sensitive diacylglycerol part was a 1-alkyl-2-acylglycerol²³. This particular diacylglycerol analogue did not affect glucose uptake, which is of course an insulin-insensitive process in hepatocytes.

The complete inhibition of diacylglycerol-stimulated glucose uptake by cytochalasin B, an inhibitor of the glucose trans-

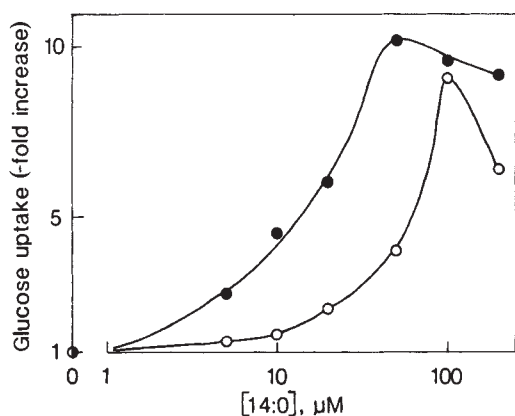


Fig. 3 Effect of diacylglycerol concentration on D-glucose uptake. Cells were incubated as described in the legend to Fig. 1 with the indicated concentrations of 1,2-ditradecanoylglycerol (14:0). Incubations were in the presence of NaTDC at 2 mM (filled circles), or in the presence of NaTDC at a concentration 20 times that of the diacylglycerol (open circles).

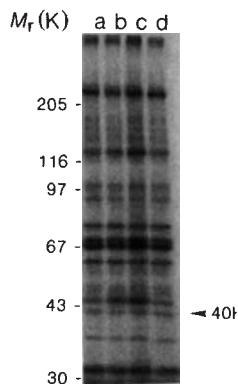


Fig. 4 Effect of diacylglycerols and phorbol ester on protein phosphorylation in cells. Cells were preincubated with 0.5 mM phosphate and 0.5 mCi ml⁻¹ of ³²P for 90 min (ref. 12) and then incubated as described in the legend to Fig. 1, but without [³H]glucose. Reactions were terminated, total cell protein prepared for SDS-polyacrylamide gel electrophoresis (8% polyacrylamide), and gels autoradiographed as described in ref. 12. Lane a, 0.1 mM 1,2-ditradecanoylglycerol + 2 mM NaTDC; b, 0.1 mM 1,2-dioctanoylglycerol + 2 mM NaTDC; c, 1 μM TPA (Sigma) + 2 mM NaTDC; d, control with NaTDC only. Identical results were obtained in the absence of NaTDC. The positions of the following marker proteins are indicated: myosin (205K), β-galactosidase (116K), glycogen phosphorylase (97K), bovine serum albumin (67K), ovalbumin (43K) and carbonic anhydrase (30K). Also indicated is the phosphoprotein of *M_r* 40K.

porter²⁴, demonstrates that the increased uptake is mediated by the glucose transporter. The extent to which transporter translocation or transporter activation participates in mediating the effect of insulin on glucose uptake is not clear³⁻⁷. After diacylglycerol treatment of cells, their plasma membranes failed to show either an increased D-glucose-inhibitable equilibrium binding of [³H]cytochalasin B (ref. 1), or an increase in [³H]cytochalasin B photolabelling²⁵ (data not shown). This indicates that the diacylglycerol acts to increase the intrinsic activity of the transporter rather than its recruitment from within the cell.

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Trans-spliced leader RNA exists as small nuclear ribonucleoprotein particles in *Caenorhabditis elegans*

Kevin Van Doren & David Hirsh

Synergen Inc., 1885 33rd Street, Boulder, Colorado 80301, USA

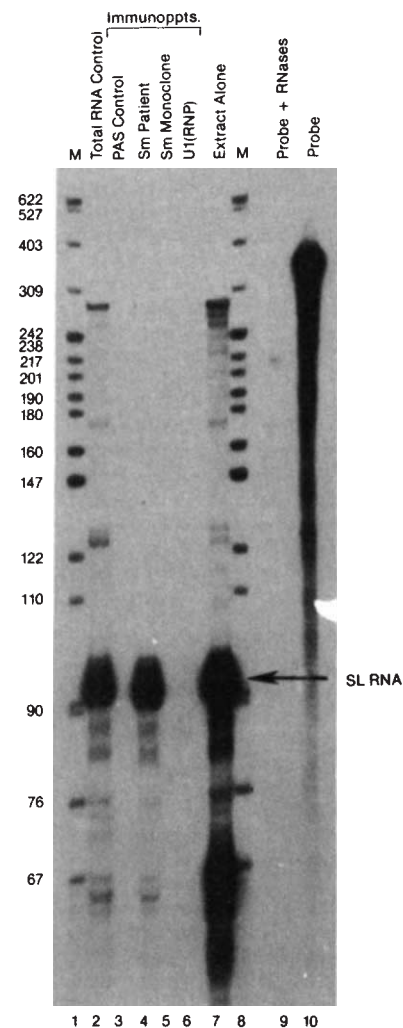
Maturation of some messenger RNAs in the nematode *Caenorhabditis elegans* involves the acquisition of a 22-base leader at their 5' ends¹. This 22-base leader, called the spliced leader (SL), is derived from the 5' end of a precursor RNA of 90-100 bases, called spliced leader RNA (SL RNA). SL RNA is transcribed from a 1-kilobase DNA repeat which also encodes the 5S ribosomal RNA. A subset of mRNAs in *C. elegans* acquire SL from SL RNA by a trans-splicing mechanism². SL behaves as a 5' exon in the trans-splicing reaction. Using antisera against the Sm antigen that is associated with small nuclear ribonucleoprotein particles (snRNPs), we precipitated SL RNA from extracts of *C. elegans*, indicating that it is bound by the Sm antigen *in vivo*. SL RNA also possesses the unique trimethylguanosine (m³g^{2,7}G) cap characteristic of most small nuclear RNAs. Therefore, SL RNA is a chimaeric molecule, made up of an snRNA attached to a 5' exon and is a constituent of a snRNP.

Trans splicing is observed in several organisms other than *C. elegans*. The best-studied are trypanosomes, in which all mRNAs mature through trans-splicing of a 35-base leader to the 5' ends^{3,4}; this 35-base spliced leader is derived from a 137-base precursor RNA^{5,6,7}. Sequence comparisons reveal little homology between the spliced leaders of *C. elegans* and trypanosomes. In contrast to trypanosomes, most mRNAs in *C. elegans* do not participate in trans-splicing. Also, mRNAs that undergo trans-splicing in *C. elegans* possess introns that are removed through cis-splicing. Cis-splicing is not found in trypanosomes.

The removal of introns from mRNA precursors (pre-mRNA) during cis-splicing occurs in large complexes, termed spliceosomes⁸⁻¹⁰, which contain a variety of snRNPs (snRNPs consist of an RNA molecule plus several proteins). A core set of proteins having determinants recognized by anti-Sm antibodies¹¹⁻¹⁵ is common to snRNPs of this class, but the RNA varies. In addition, Sm snRNPs can have one or more unique proteins¹¹⁻¹⁵.

Fig. 1 Immunoprecipitation of SL RNA from cell-free extracts of *C. elegans*. The autoradiogram shows protection against degradation by RNase A and RNase T1 of 32 P-labelled, antisense SL RNA synthesized *in vitro*, by RNA immunoprecipitated from cell-free extracts. Each lane of the gel contains the RNA immunoprecipitated from an amount of extract containing 1.5 μ g total RNA. The band representing SL RNA is marked by the arrow. Lanes 1 and 8 are the labelled products of a *Hpa*II digest of pBR322 used as relative molecular mass standards. Lane 10 is the 32 P-labelled antisense RNA probe. Lane 9 shows the same antisense RNA probe following treatment with RNases A and T1. Lanes 2–6 show protection of the 32 P-labelled antisense RNA by RNA isolated from cell-free extracts before immunoprecipitation (lane 2), or by RNA immunoprecipitated using protein A-Sepharose (lane 3), patient serum anti-Sm/JE (lane 4), monoclonal antibody Y-12 (lane 5), or anti-(U1)RNP antiserum (lane 6). Lane 7 is a control showing protection of the probe RNA by 5 μ g total RNA purified from *C. elegans*.

Methods. The plasmid used for *in vitro* transcription of the antisense SL RNA contained a 245-base pair (bp) *Bam*HI/*Bst*UI fragment from a cloned copy of the 1-kilobase (kb) DNA repeat²⁶ containing the 5S and the SL RNA genes (gift from Barry Honda). Plasmid DNA was linearized for transcription. Transcription reactions were performed essentially as described by Green *et al.*²⁷, except that the reaction mixtures were 20 μ l and contained 2 mM spermidine, 5 μ M CTP, 250 μ M m^7 GpppG, 40 units of RNasin, 60 μ Ci [32 P]CTP and 5–10 units T7 polymerase. Incubation was at 37 °C for 1 h and was followed by treatment with RNase-free DNase for 10 min. The RNA was extracted with phenol:chloroform (1:1, v/v), ethanol precipitated, purified by electrophoresis through a 5% polyacrylamide thin sequencing gel, eluted, ethanol precipitated and redissolved in RNase-free, glass-distilled water. Cell-free extracts were prepared from embryos. Embryos (3×10^5 per ml) were suspended in 150 mM NaCl, 50 mM Tris (pH 7.4), 0.05% Nonidet P-40 nonionic detergent (NP40) and passed twice through a French press at 68.9 megapascals. Insoluble material was removed by centrifugation at 12,000g for ten min at 4 °C. The supernatant was incubated at 4 °C for 90 min with antibody complexed to protein A-Sepharose. The protein A-Sepharose/antibody/antigen complex was pelleted by centrifugation in a microfuge for 10 s. The supernatant was removed and the pellet washed four times with 1 ml of 500 mM NaCl, 10 mM Tris (pH 8.0) and 0.1% NP40. The final pellet was resuspended in 300 μ l of 150 mM NaCl, 50 mM Tris (pH 7.4) and 0.05% NP40. 10% SDS (30 μ l), 30 μ l of 2 M sodium acetate (pH 5.2) and 350 μ l of phenol:chloroform (1:1, v/v) were added. The RNA was extracted and ethanol-precipitated. RNA was redissolved in RNase-free, glass-distilled water and hybridized to the 32 P-labelled antisense SL RNA probe as described by Zinn *et al.*²¹. The RNase protection was carried out as described²¹. Protected RNAs were analysed by electrophoresis through thin sequencing gels, 7 M in urea and 9% in polyacrylamide, followed by autoradiography.



By analogy with *cis*-splicing, we expected *trans*-splicing to occur in spliceosomes and therefore that the SL RNA would be associated with snRNPs.

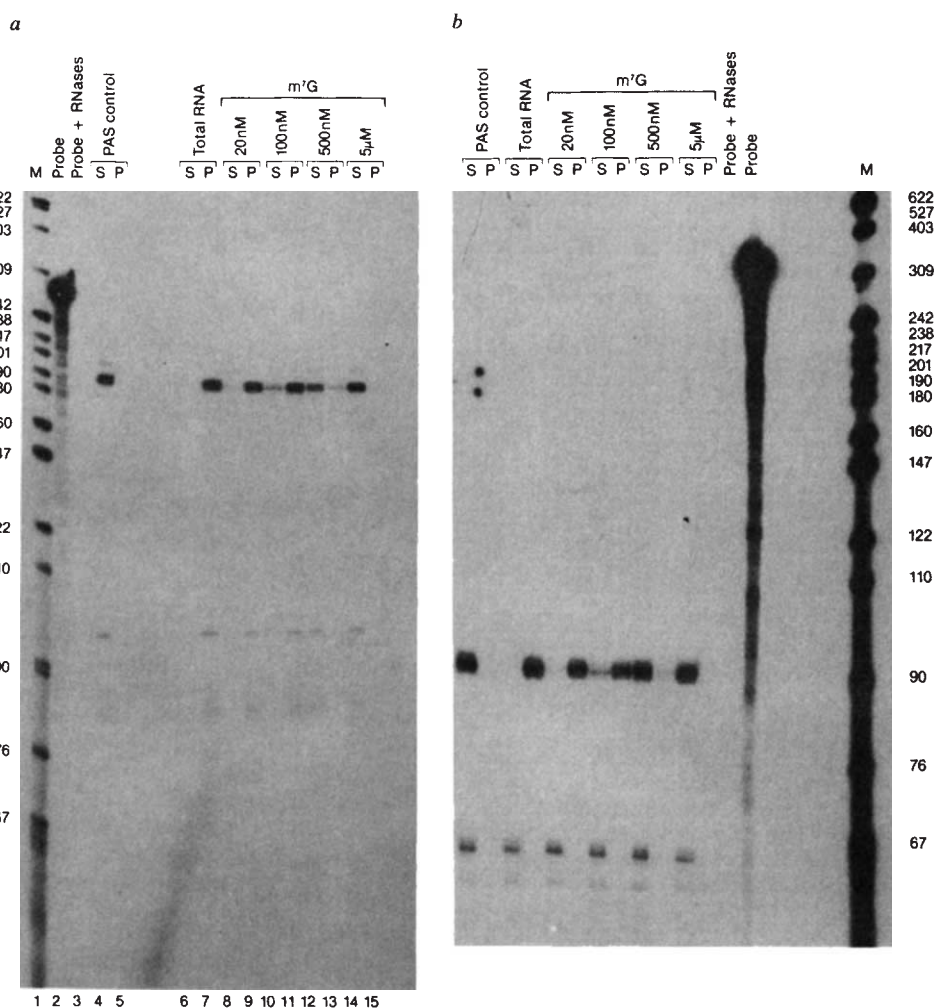
Bruzik *et al.*, in this issue, show computer-generated possible secondary structures of spliced leader precursors from different organisms that undergo *trans*-splicing¹⁶. Among several conserved features is an Sm-binding consensus sequence in a single-stranded region between two helices. The presence of this sequence suggests that the SL RNA in *C. elegans* is bound to the highly conserved Sm protein antigen^{17–19}. To determine whether the proposed Sm binding site is functional, we assayed cell-free extracts of *C. elegans* for the presence of the SL RNA in anti-Sm-precipitable material. Immunoprecipitations were carried out using serum from patients with the autoimmune disease systemic lupus erythematosus (SLE), which contains anti-Sm antibodies²⁰. RNA in the immunoprecipitates was identified by hybridization to a 32 P-labelled antisense SL RNA; labelled probe in hybrids was then protected against RNase A and RNase T1 degradation²¹. One patient's serum (anti-Sm/JE) precipitated SL RNA from extracts of *C. elegans*, whereas a monoclonal antibody (Y-12) failed to precipitate SL RNA (Fig. 1). Sera from three additional patients (anti-Sm/AM, anti-Sm/SM and anti-Sm/SK) also precipitated SL RNA from cell-free extracts of *C. elegans* (data not shown). We believe that the consensus Sm binding site found in SL RNA binds Sm antigen, rendering SL RNA immunoprecipitable by anti-Sm patient sera, although we cannot exclude the possibility that SL RNA is immunoprecipitated through association with another anti-Sm precipitable RNA. Total RNA isolated directly from worms by guanidine isothiocyanate extraction contains, in addition to the full-length protected RNA, a smaller RNA fragment

approximately the size expected for the SL RNA without its 22-base spliced leader. We believe that this fragment is derived from the Y-branched intermediate². The absence of this smaller RNA in samples from extracts is probably due to endogenous nucleases in the immunoprecipitation reaction mixtures. Anti-(U1)RNP does not precipitate SL RNA from extracts of *C. elegans* (Fig. 1); this anti-(U1)RNP precipitates U1 snRNP from cell-free extracts of *C. elegans* (data not shown). Therefore, proteins specific to the (U1)RNP are not among those bound to the SL RNA.

The presence of SL RNA in anti-Sm-precipitable particles indicates that SL RNA in *C. elegans* is functionally homologous to the snRNAs used for *cis*-splicing. In addition to an Sm binding site, another feature of many snRNAs is the unique cap structure, $m_3^{2,7}$ G (refs 22, 23). Therefore, we determined whether SL RNA from *C. elegans* also has an $m_3^{2,7}$ G cap (Fig. 2). A monoclonal antibody (RL; ref. 24), specific for $m_3^{2,7}$ G, and two additional anti- $m_3^{2,7}$ G antibodies, K121 and Fr3, all immunoprecipitate SL RNA from total RNA purified from *C. elegans*. RNA of the size expected for the debranched intermediate is observed in the supernatant, demonstrating that this RNA fragment does not have an $m_3^{2,7}$ G cap. This would be expected if it is the SL RNA without its 5' SL sequence.

Antibodies to $m_3^{2,7}$ G cross-react to some extent with (m^7 G). To check that immunoprecipitation of SL RNA was due to reactivity with $m_3^{2,7}$ G and not with m^7 G, we compared immunoprecipitations of SL RNA with *C. elegans* U2 RNA in the presence of m^7 G as a competitor. U2 RNA is an snRNA known to have an $m_3^{2,7}$ G cap in every system examined. SL RNA and U2 RNA are immunoprecipitated identically by anti- $m_3^{2,7}$ G antibody in the presence of increasing concentrations

Fig. 3 m^7G as a competitor in the immunoprecipitations by anti- $m_3^{2,7}G$ antibodies. **a**, Autoradiogram of RNA immunoprecipitated by anti- $m_3^{2,7}G$ antibodies analysed by RNase protection. The probe used in this experiment is an antisense transcript of the *C. elegans* U2 gene. Lane 1 shows the products of *Hpa*II digest of pBR322 used as relative molecular mass standards. Lanes 2 and 3 show the antisense RNA probe before (1:10 dilution) and after treatment with RNases, respectively. Lanes 4 and 5 are the supernatant and precipitate from 10 μ g total RNA when protein A-Sepharose is added without antibody. Lanes 6 and 7 are supernatant and precipitate from 10 μ g of total RNA. Lanes 8–15 are pairs of supernatants and precipitates from 10 μ g of total RNA in the presence of increasing concentrations of m^7G designated in the figure. **b**, Autoradiogram of an RNase protection using the same RNA as in **a**, using an antisense SL RNA as probe. Lanes 1 and 2 are the supernatant and precipitate when protein A-Sepharose is used without antibody. Lanes 3 and 4 are the supernatant and precipitate from 10 μ g total RNA. Lanes 5–12 are pairs of supernatants (S) and precipitates (P) from 10 μ g total RNA in the presence of increasing concentrations of m^7G . **Methods.** Immunoprecipitation, hybridization and RNase protection were performed as described in Fig. 2, except that some samples contained m^7G as a competitor at the concentrations indicated. The anti- $m_3^{2,7}G$ antibody used in these experiments was K121.



of m^7G (Fig. 3). This indicates that U2 RNA and SL RNA have the same cap structure, namely $m_3^{2,7}G$.

Taken together, these results lead us to conclude that the SL RNA exists *in vivo* as an RNP particle, called SL snRNP. This particle performs *trans*-splicing but not *cis*-splicing. Therefore we propose that there are separate *cis*- and *trans*-spliceosomes, depending on whether they contain a U1 snRNP or an SL snRNP.

SL RNA is unique in that it contains a 5' exon as part of an snRNA and the exon is transferred from the SL RNA to the pre-mRNA. Therefore, SL RNA represents the first example of an snRNA that is consumed during the splicing reaction. Because SL RNA acts stoichiometrically, higher levels of SL RNA are required than would be necessary for a catalytic role. This may explain why there are 110 copies of the SL RNA genes in *C. elegans*¹. The steady-state levels of SL RNA are lower than those of U2 RNA (unpublished results). This could be due to a very rapid turnover of newly synthesized SL RNA through the *trans*-splicing reaction, coupled with the fact that only 10% of the mRNAs in *C. elegans* mature through *trans*-splicing²⁵. As the 5' portion of SL RNA is transferred to pre-mRNA, it would seem reasonable that the fate of the 3' portion is equivalent to that of an intron from a *cis*-spliced pre-mRNA, but there remains the possibility that it serves a unique function.

The 5' end of SL RNA possesses the $m_3^{2,7}G$ cap structure characteristic of snRNAs. The transfer of the 5' exon of SL RNA, including the $m_3^{2,7}G$ cap, leads us to predict that *trans*-spliced mRNAs in *C. elegans* will possess an $m_3^{2,7}G$ cap. It will be interesting to determine if mRNAs in *C. elegans* possess

such a cap structure and if they do, whether they can be translated. If mature *trans*-spliced mRNAs do not possess a $m_3^{2,7}G$ cap, then a novel processing reaction must follow *trans*-splicing.

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Note added in proof: J. Thomas *et al.* (*Cell* **54**, 533–539, 1988) here also found that SL RNA is precipitable by anti-Sm and anti- $m_3^{2,7}G$ antibodies. We are grateful to Jeff Thomas and Tom Blumenthal for communicating their results prior to publication.

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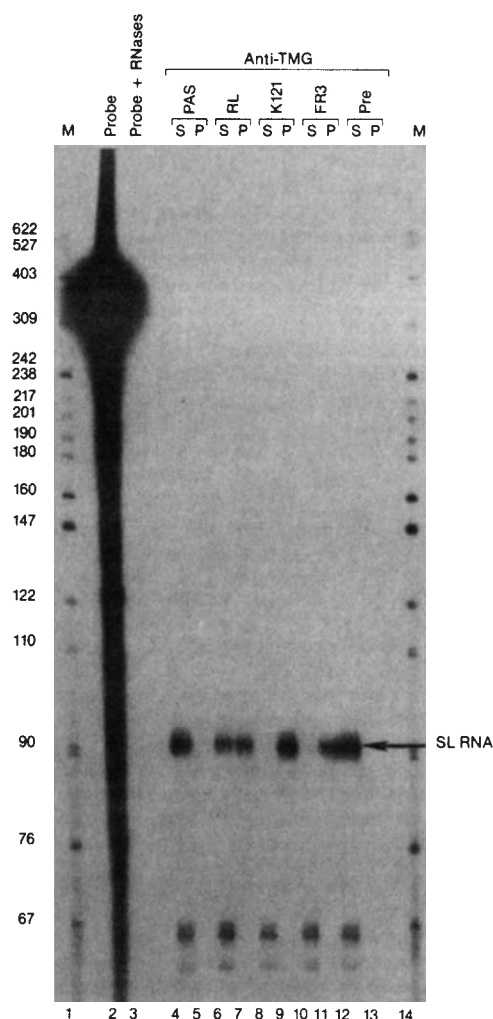


Fig. 2 Analysis of RNA immunoprecipitated from purified total *C. elegans* RNA by anti- $m_{3.2.7}G$ antibodies. The autoradiogram shows protection of an *in vitro* synthesized, ^{32}P -labelled antisense SL RNA by RNA immunoprecipitated by anti- $m_{3.2.7}G$ antibodies. The band representing SL RNA is indicated by the arrow. Lanes 1 and 14 are the labelled products of a *Hpa*II digest of pBR322 used as relative molecular mass standards. Lanes 2 and 3 show the antisense RNA probe before and after treatment with RNases, respectively. Lanes 4 and 5 are the supernatant and precipitate resulting when protein A-Sepharose is added to total RNA without antibody. Lanes 6–11 are pairs of RNase protection products of RNA from either the supernatant (S) or the precipitate (P) using three different anti- $m_{3.2.7}G$ (TMG) antibodies: lanes 6 and 7, RL antibody; lanes 8 and 9, K121 antibody; lanes 10 and 11, FR3 antibody. Lanes 12 and 13 show that pre-immune serum to the FR3 anti- $m_{3.2.7}G$ antibody shown in lanes 10 and 11 does not react to the $m_{3.2.7}G$ cap.

Methods. Immunoprecipitation, hybridization and RNase protection were performed as described in Fig. 1, with the exception that 10 μ g purified total RNA from *C. elegans* in 300 μ l 150 mM NaCl, 50 mM Tris (pH 7.4), 0.05% NP40 was used instead of cell-free extract. Antibody RL was prepared by R. Lührman²⁴ and provided by N. Agabian; K121 was provided by Adrian Krainer and FR3 by Doug Black. K121 is an immunoglobulin G1 monoclonal that does not bind protein A (A. Krainer, personal communication). Therefore, heavy-chain specific, affinity-purified, rabbit anti-mouse IgG (Zymed, numbers 61–6,000) was included to bind K121 to protein A-Sepharose.

Trans splicing involves a novel form of small nuclear ribonucleoprotein particles

James P. Buzik*, Kevin Van Doren†, David Hirsh† & Joan A. Steitz*

* Howard Hughes Medical Institute, Department of Molecular Biophysics and Biochemistry, Yale University, 333 Cedar Street, New Haven, Connecticut 06510, USA

† Synergen Inc., 1885 33rd Street, Boulder, Colorado 80301, USA

The *trans*-splicing reaction occurring in trypanosomes and related species^{1–4} as well as in the nematode *Caenorhabditis elegans*⁵ involves the transfer of a 5' exon from a spliced leader transcript (SL RNA) to a precursor messenger RNA transcript with a 3' splice acceptor site. This seems to take place in the same nuclear compartment as normal *cis* splicing and proceeds through Y-branched intermediates^{6,7} analogous to the lariats formed in *cis* splicing. The cellular machinery catalysing *cis* and *trans* splicing^{8,9,10} might therefore be expected to share some components, particularly in the nematode where some mRNAs are produced by both *cis* and *trans* splicing⁵. We generated possible secondary structures for the SL RNAs of several species and found they were remarkably similar although neither nucleotide sequence nor length is conserved. Each contained three stem-loops; strikingly the 5' splice site is adjacent to the turn of the most 5' loop and an Sm-binding consensus sequence¹¹ is found between the second and third stem-loops. Sm is an antigen associated with small nuclear ribonucleoprotein particles (snRNPs). When incubated in HeLa cell nuclear extracts, SL RNAs become immunoprecipitable by anti-Sm, but not by other autoantibodies directed against proteins of mammalian snRNPs^{12,13}. We propose that SL RNAs have a dual function in the *trans* splicing process: they consist of a 5' exon covalently linked to an snRNA-like sequence and seem likely to exist as Sm snRNP particles (SL snRNPs) within the cell. Just as the RNA in the U1 snRNP base-pairs with the 5' splice site^{14,15}, rendering it susceptible to attack in the *cis*-splicing reaction¹⁶, so might the SL snRNP autonomously activate its own 5' splice site and thereby eliminate the need for a U1-like snRNP in the *trans*-splicing machinery.

If SL RNAs do function as both 5' exon and snRNA in the *trans*-splicing reaction, then we could expect either their sequences, their secondary structures, or both to be conserved. Comparison of seven SL RNAs reveals only limited sequence similarity. In trypanosomes and related species, identical regions are concentrated within the 5' exons (39–41 bases) and extend several bases 3' to the splice site; the nematode 5' exon sequence (22 bases) bears little similarity to the others. But there is a sequence resembling the Sm-binding site consensus in the 3' portion of each SL RNA. Sm epitopes characterize certain proteins¹³ common to the snRNPs involved in precursor mRNA (pre-mRNA) splicing^{9,10,17} and other RNA processing events in eukaryotic cell nuclei. Reconstitution studies have shown that a sequence of R-A-(U)_n-G-R is critical for the binding of these proteins; this region is single-stranded and is followed by a stem-loop structure in well characterized U RNAs¹¹. In our derivation of structures for the SL RNAs we therefore included the constraint that the Sm binding sequence be single-stranded (Fig. 1). We found that each SL RNA can be folded into three stem-loops occupying roughly equivalent positions in the primary structure. Within the first stem-loop in all seven cases, the cleavage site separating the 5' exon from the remainder of the SL RNA resides between two base-paired G residues, the first of which closes the loop. Within the 3' portion of each SL RNA, the Sm binding sequence is flanked by two stem-loops, but they are of different length and sequence in the different SL RNAs. The structures of Fig. 1 suggest that the 3' region of each SL RNA resembles a U RNA which may be assembled *in vivo* into a snRNP-like structure (SL snRNP).

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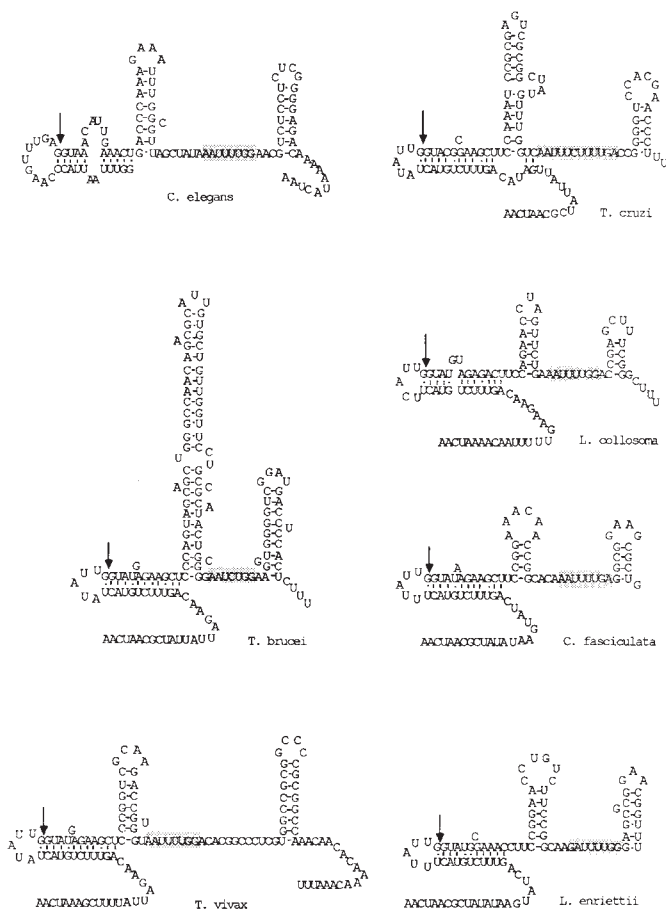


Fig. 1 Possible secondary structures of SL RNAs. The sequences are taken from the following references: *T. brucei*¹, *T. cruzi*², *T. vivax*², *L. enriettii*³, *L. collosoma*¹, *C. fasciculata*⁴ and *C. elegans*⁵ (note that an extra G residue has been inserted at position 53, as a result of additional analysis). Arrows indicate the 5' splice sites and shaded boxes cover the Sm binding sites. (Note that C residues are tolerated within the U-stretch of Sm-binding sites in some organisms, notably *Xenopus*^{11,20}.) Overall lengths of SL RNAs range from ~90 to ~140; the 3' ends of many of these transcripts have not been precisely mapped. The Fold program, version 5.0 from the University of Wisconsin Genetics Computer Group, was used as a guide in devising these structures. ΔG values were calculated both for the above and for 'lowest free energy' structures generated with no constraints; in each case except *L. enriettii* and *C. fasciculata*, the two ΔG values were within 10% of each other. The ΔG (kcal/mole) values are: -17.1 and -16.0, *C. elegans*; -25.9 and -24.1, *T. cruzi*; -14.3 and -17.5, *L. collosoma*; -29.2 and -26.5, *T. vivax*; -44.9 and -42.3, *T. brucei*; -16.9 and -12.5, *C. fasciculata*; and -21.2 and -6.4, *L. enriettii* for the unconstrained and presented structures, respectively.

To determine whether the Sm binding sequences were functional we synthesized SL RNAs *in vitro* and tested their ability to associate with Sm proteins on incubation in a HeLa cell nuclear extract¹⁸. Binding can easily be assessed by immunoprecipitability on subsequent addition of anti-Sm antibodies. The first SL RNA we examined was from the nematode *C. elegans*: it became efficiently immunoprecipitable with anti-Sm autoantibodies from four patients, as well as with the Y-12 monoclonal¹⁹ (Fig. 2a, lanes 1-5). Control reactions, including anti-(U2)RNP, anti-(U1)RNP, and non-immune sera (lanes 6-9), all proved negative. When the antisense strand of the SL RNA was incubated in the same extract, no immunoprecipitability was seen with any of the antibodies tested (lanes 12-20), except for occasional inefficient immunoprecipitation given by a patient anti-Sm sera (lane 12). The SL RNA of *Leptomonas collosoma* was synthesized next and also became efficiently

immunoprecipitable by patient anti-Sm sera as well as by the monoclonal anti-Sm antibody after incubation in HeLa cell nuclear extract (Fig. 2b, lanes 1-3). Since a second Sm binding sequence is present near the 5' end of the *L. collosoma* SL RNA, we altered this sequence by changing uridine residues in positions 12-16 to adenosines (*L. collosoma*-5' Sm); efficient immunoprecipitation by anti-Sm antibodies was retained (Fig. 2b, lanes 18-20), indicating that the Sm sequence between the second and third stem loops is the functional binding site. We also tested the SL RNA from *Trypanosoma cruzi* (Fig. 2c, lanes 1-4), but neither it nor its antisense version gave a detectable immunoprecipitate. We suspected that this was due to a single cytidine within the Sm sequence (AAUUUCUUUUGA), given that none of the many known mammalian U RNAs of the Sm class has such an interruption²⁰; the single reported exception, U8 (ref. 20) is not anti-Sm precipitable in our hands (K. Tyc, unpublished observations). We therefore constructed a mutant *T. cruzi* SL RNA with the cytidine in the Sm sequence deleted (*T. cruzi* ΔC); it became immunoprecipitable at a level comparable to that of the other SL RNAs (Fig. 2c, lanes 6-8), indicating that cytidine residues are not tolerated in human Sm binding sites. Also, the antisense transcript of *T. cruzi* has what appears to be an excellent mammalian Sm sequence (GAUUUGG); its lack of activity (Fig. 2c, lane 1) confirms previous indications that the context of an Sm sequence is important for proper binding of Sm proteins¹¹. Finally, we synthesized the SL RNA of *Crithidia fasciculata*; upon incubation it also became precipitable with the monoclonal anti-Sm antibody (Fig. 2c, lane 14). Thus, as predicted by the structures of Fig. 1, HeLa cell Sm proteins bound each of three different SL RNAs tested, as well as a mutant *T. cruzi* SL RNA from which the cytidine residue within the Sm binding site sequence had been removed.

It was possible that SL RNAs might also bind U1-specific proteins since base-pairing across the 5' splice site is provided by the U1 snRNP in *cis*-splicing^{14,15}. But we did not detect any immunoprecipitability of an SL RNA transcript with anti-(U1)-RNP after incubation in the HeLa cell nuclear extract (Fig. 2a, lane 7; 2b, lanes 5 and 22; 2c, lanes 10 and 16). We therefore tested an SP6 transcript of U1 RNA in our extract. It became immunoprecipitable with a patient anti-(U1)RNP serum (Fig. 2d, lane 3), as well as with anti-Sm antibodies (lanes 1 and 2), showing that our assay would have detected the assembly of the SL RNA with at least one protein carrying specific U1 RNP epitopes if the correct signals were present in the SL RNAs.

We predicted, on the basis of these results (Fig. 2), that SL RNAs would be bound by Sm proteins *in vivo*. This has been borne out in the case of the nematode SL transcript. Two groups^{21,22} have observed that SL RNAs in nematode cell extracts are precipitable by certain patient (but not by the Y-12 monoclonal¹⁹) anti-Sm antibodies. Likewise, the *Saccharomyces cerevisiae* snRNAs that are involved in splicing specifically bind *Xenopus* Sm proteins on injection into oocytes or eggs²³, but are precipitated from yeast cell extracts only with certain patient anti-Sm antibodies²⁴. In trypanosomes and related species, no cross-reaction with any anti-Sm antibody has been reported, but SL RNAs have been observed to be assembled into RNP particles^{25,26}. The presence in all SL RNAs of a sequence resembling an Sm binding site is good evidence that trypanosomes do contain functionally equivalent (albeit antigenically distinct) Sm proteins.

If the biogenesis of SL RNAs resembles that of U RNAs in vertebrate cells, SL RNAs would be transcribed by RNA polymerase II and then rapidly exported to the cytoplasm where, by association with Sm proteins, they would create a nuclear re-entry signal absent in the RNA or proteins alone^{11,27}. Indeed, trypanosome SL RNAs have been shown to be synthesized by RNA polymerase II (ref. 28). Likewise, the *C. elegans* SL RNA gene⁵, which also seems to be transcribed by RNA polymerase II (M. Golumb and S. Bektesh, personal communication) possesses special sequence elements characteristic of U RNA

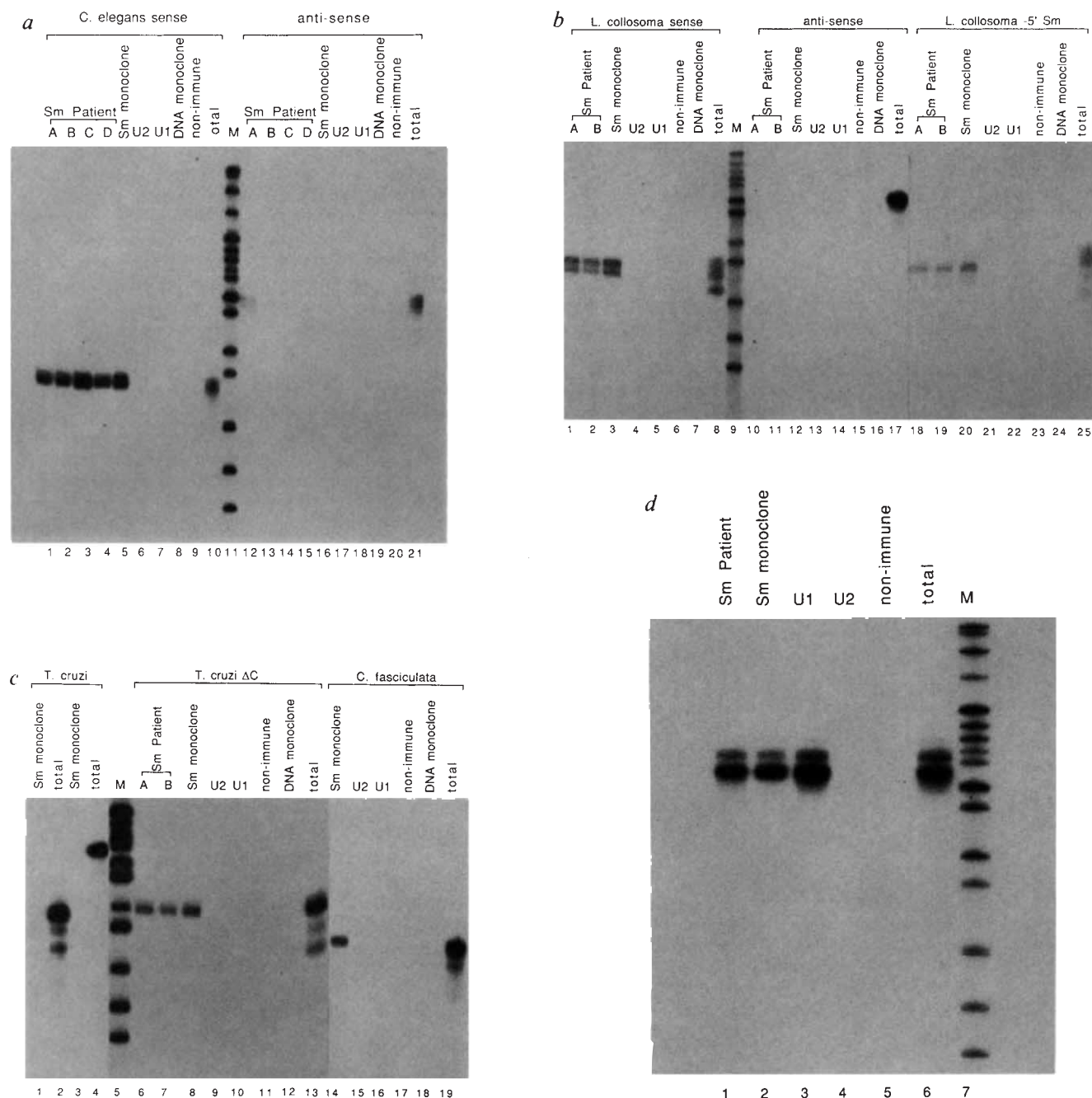


Fig. 2 Assembly of SL snRNPs in HeLa cell nuclear extracts. *a*, *C. elegans* SL RNA in the sense (lanes 1–10) and the antisense (lanes 12–21) orientations. One-fifth of the total RNA from a reconstitution aliquot was loaded in the total lanes; therefore we estimate a binding efficiency of ~40% for Sm proteins. *b*, *L. collosoma* SL RNA in the sense (lanes 1–8) and antisense (lanes 10–17) orientations. Lanes 18–25 show the *L. collosoma* -5' Sm transcript. *c*, Anti-Sm (Y-12 monoclonal) precipitates (lanes 1 and 3) and the totals (lanes 2 and 4) of the *T. cruzi* SL RNA in both the sense (lanes 1 and 2) and antisense (lanes 3 and 4) orientations. Lanes 6–13 show the *T. cruzi* ΔC construct. Lanes 14–19 show the *C. fasciculata* transcript. In *b* and *c*, two-fifths of the total RNA from a reconstitution aliquot was loaded in the total lanes; therefore we estimate Sm binding efficiencies of ~40% for the wild-type and mutant *L. collosoma*, the mutant *T. cruzi* and the *C. fasciculata* SL RNAs. *d*, Assembly of a human U1 RNA transcript. The total lanes contain RNA from a full aliquot of the reaction; therefore the reconstitution is quantitative. M lanes are pBR322 *Hpa*II labelled DNA size markers.

Methods. Reconstitution conditions were as described¹⁸ except that nuclear extract³⁷ was used. Cytoplasmic extracts yielded comparable results (data not shown). Immunoprecipitations were performed on 16 μ l aliquots of a reaction containing 10^5 c.p.m. (Cerenkov) of ³²P-labelled gel-purified transcripts by adding 10 μ l of the indicated antibodies, pre-bound to 2.5 mg protein A-Sepharose and incubating for 1 h at 4°C. Pellets were washed five times with IPP (0.5 M NaCl, 1% nonidet-P40 and 0.01 M Tris, pH 8.0), PCA (phenol:chloroform:isoamyl alcohol, 50:49:1)-extracted and ethanol precipitated. RNAs were resolved on a 10% polyacrylamide 7 M urea gel and visualized by autoradiography. Anti-Sm sera used were the Y-12 monoclonal¹⁹ and the following patient sera: A, Sm JE; B, Sm AM; C, Sm SK; D, Sm SM. The T7 SL RNA transcripts (Boehringer protocol) each contained extra bases at their 5' and 3' ends. The 116-base *C. elegans* sense transcript has 9 and 6 extra bases respectively and migrates between the 90 and 110 base-markers. The antisense (150 bases) migrates between the 146 and 160 markers. The 109-base *L. collosoma* sense transcript has 2 and 9 extra bases, respectively, and migrates between the 90 and 110 markers. The antisense (178 bases) migrates between the 160 and 180 markers. The 117-base *L. collosoma* -5' Sm transcript has 2 and 15 extra bases respectively and migrates between the 110 and 122 markers. The 124-base *T. cruzi* sense transcript has 2 and 7 extra bases, respectively, and migrates between the 110 and 122 base markers. The antisense (191 bases) migrates between the 160 and 180 markers. The 123-base *T. cruzi* ΔC transcript has 2 and 7 extra bases, respectively, and migrates between the 110 and 122 markers. The 100-base *C. fasciculata* transcript has 2 and 7 extra bases respectively, and migrates between the 90 and 110 markers. The human U1 clone (provided by T. Pederson) yielded a 189-base SP6 transcript with the correct 5' end and 25 extra 3' bases; it migrates between the 160 and 180 base markers.

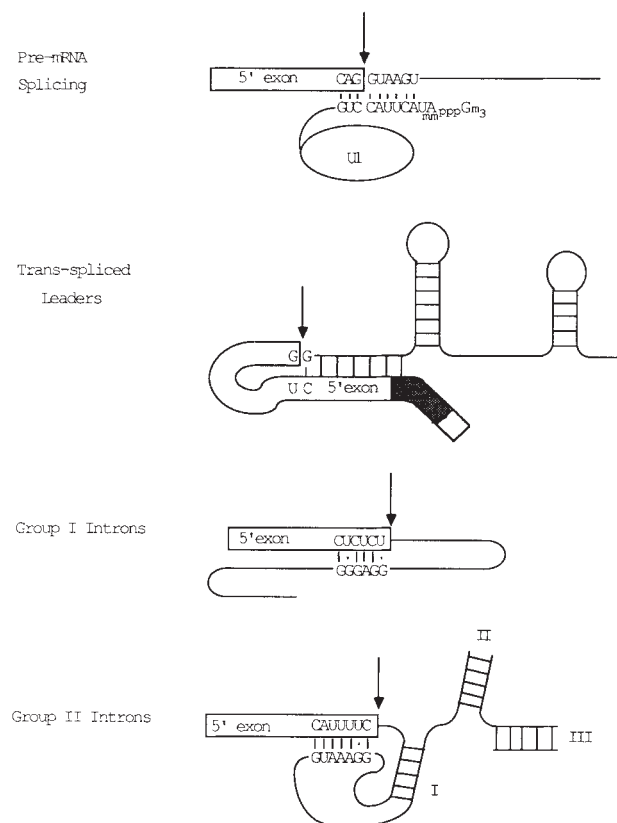


Fig. 3 RNA-RNA base-pairing at 5' splice sites. Exons are indicated by boxes and introns by lines; arrows designate 5' splice sites. The top panel depicts the 5' consensus sequence of a pre-mRNA interacting with the complementary 5' end of U1 snRNA in a U1 snRNP^{14,15}. The second panel shows an SL RNA, illustrating base-pairing across the 5' splice site (the G-C base-pair appears in all SL RNAs; 6 out of 7 SL RNAs have a G-U base-pair immediately preceding the 5' splice site, the seventh is a G-C). The shaded portion of the exon indicates the region in trypanosome SL RNAs that is complementary to U2 snRNA³². The third panel shows the interaction between the 5' exon and the internal guide sequence of a group I intron (sequences from the *Tetrahymena* ribosomal RNA self-splicing intron²⁹). The bottom panel depicts a group II self-splicing intron, showing a proposed interaction between the exon and intron binding sites (sequences from intron a5 of the yeast cytochrome oxidase subunit I (ref. 31), drawn in a slightly different configuration).

genes: an enhancer at -304, a TATA-like sequence at -52 and a 3' end-formation signal at +13, deviating in only two, one and three positions, respectively, from the vertebrate consensus sequences²⁷. The ability of SL RNAs to associate with Sm proteins suggests that *trans* splicing, like *cis* splicing, occurs in the nucleus. Thus, multiple steps in the biogenesis of SL snRNPs could provide additional mechanisms for regulating the production of mRNAs that acquire *trans*-spliced leaders.

It has been proposed that, in all types of splicing processes except for transfer RNA splicing, base-pairing across or adjacent to the 5' splice site activates the phosphodiester bond for the first step of the reaction (Fig. 3). In both group I and group II self-splicing reactions²⁹, intron sequences provide the complementarity^{30,31}; in snRNP-mediated pre-mRNA splicing, the 5' end of the U1 snRNA base-pairs with the 5' splice site^{14,15}; in the SL RNAs, we suggest that upstream exon sequences are involved. Exon sequences of trypanosome SL RNAs have previously been proposed to base-pair with U2 snRNA^{26,32}; there are complementary sequences between bases 17 and 23 of the *T. brucei* SL RNA (the position in others varies slightly) and in the completely conserved portion of the single-stranded region (bases 40 to 46) between the first two stem-loop structures of

the U2 molecule²⁰. Such an intermolecular interaction would not disrupt the intramolecular structures shown in Fig. 1. Instead, SL RNA-U2 base-pairing would extend the helix of the first SL RNA stem-loop (see the shaded exon region in Fig. 3); and, if U2 also interacts with the acceptor RNA (as it does in yeast *cis* splicing³³) the branch point would be brought close to the 5' splice site of the SL RNA. Such a trimolecular model for the first step of *trans* splicing differs greatly from that proposed recently where the 5' splice site of the SL RNA pairs directly with the branch point of the acceptor RNA³⁴. The association of individual transcripts to form extensive secondary structures typical of group II introns has also been proposed for a different variety of *trans* splicing occurring in chloroplasts³⁵.

It is possible that the fusion of a 5' exon with an snRNA in SL RNAs represents a remnant of the evolutionary progression from group II self-splicing reactions to snRNP-mediated pre-mRNA splicing. If SL snRNPs function as both 5' exon and U1 snRNP in the *trans*-splicing reaction, this would explain why a U1 snRNA has not been identified in trypanosomes (refs 32 and 36; C. Tschudi and E. Ullu, personal communication). Accordingly, U1 snRNPs in nematodes should participate only in *cis*-splicing. It will be interesting to discover whether nematodes possess two kinds of U2 (and other) snRNAs, one for *cis* and one for *trans* splicing. Finally, the association of SL RNAs with the Sm antigen suggests strategies for identifying potential spliced leader sequences on the mRNAs of other eukaryotic organisms.

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GAL4-VP16 is an unusually potent transcriptional activator

Ivan Sadowski*, Jun Ma*, Steve Triezenberg† & Mark Ptashne*

* Department of Biochemistry and Molecular Biology, Harvard University, 7 Divinity Avenue, Cambridge, Massachusetts 02138, USA

† Department of Biochemistry, Michigan State University, East Lansing, Michigan 48824, USA

Recent work has defined a class of transcriptional activators¹⁻⁵, members of which activate transcription in yeast, plant, insect and mammalian cells⁶⁻⁹. These proteins contain two parts: one directs DNA binding and the other, called the activating region, presumably interacts with some component of the transcriptional machinery. Activating regions are typically acidic and require some poorly-understood aspect of structure, probably at least in part an α -helix^{1-5,10}. Here we describe a new member of this class, formed by fusing a DNA-binding fragment of the yeast activator GAL4 to a highly acidic portion of the herpes simplex virus protein

VP16 (ref. 11; also called Vmw65). VP16 activates transcription of immediate early viral genes by using its amino-terminal sequences to attach to one or more host-encoded proteins that recognise DNA sequences in their promoters¹¹⁻¹⁵. We show that the hybrid protein (GAL4-VP16) activates transcription unusually efficiently in mammalian cells when bound close to, or at large distances from the gene. We suggest that the activating region of VP16 may be near-maximally potent and that it is not coincidental that such a strong activator is encoded by a virus.

The experiments shown in Fig. 1 were performed by transfecting Chinese hamster ovary (CHO) cells with an 'effector' plasmid encoding GAL4 or one of its derivatives and a 'reporter' plasmid bearing the chloramphenicol acetyl transferase (CAT) gene fused to the mouse mammary tumour virus long terminal repeat (MMTV-LTR) (Fig. 1a). Reporter plasmids are distinguished by the presence (or absence) of insertions of the yeast sequence UAS_G, that contains four GAL4 binding sites¹⁶. GAL4-VP16 comprises the first 147 amino acids of GAL4 fused to the carboxyl-terminal 78 amino acids of VP16. These VP16 residues are highly acidic (net charge -18) and are necessary for transcriptional activation by VP16 (ref. 11).

In a HeLa cell assay (Fig. 1b) with a reporter bearing a UAS_G just upstream of the TATA sequence (that is at position -110), GAL4-VP16 elicited about 100-fold more activity than either

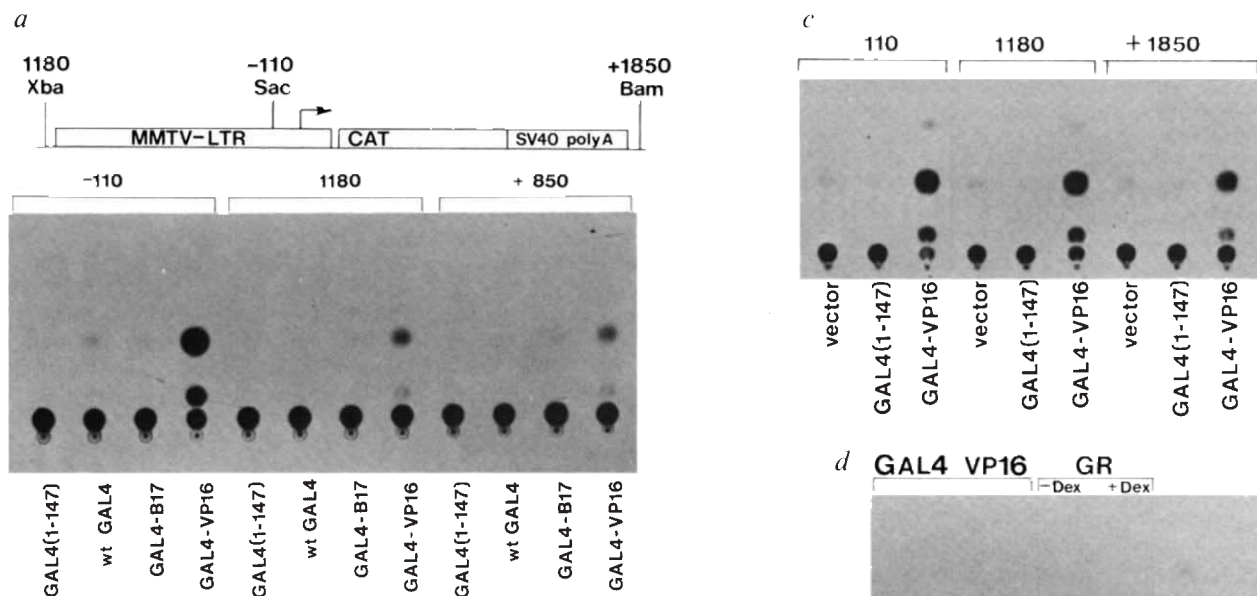


Fig. 1 CAT activity resulting from activation of transcription by GAL4 derivatives. *a*, The reporter gene used in these experiments has the MMTV-LTR fused to the bacterial chloramphenicol acetyl transferase (CAT) gene. A 365-base-pair (bp) fragment from yeast UAS_G was cloned into the XbaI, SacI, or BamHI sites to position GAL4 binding sequences at -1180, -110 and +1850 nucleotides (pMCU-1180, pMCU-110 and pMCU+1850 respectively) from the transcriptional start site. The pMC plasmid carries the MMTV-CAT fusion gene with no insertions. *b*, HeLa cells were co-transfected with effector plasmids, which express GAL4 derivatives and reporter constructs bearing a UAS_G at -110, -1180 and +1850 nucleotides from the transcriptional start site (wt, wild-type). *c*, CHO-DUKX (ref. 6) cells were co-transfected with the effector and reporter constructs as described above. *d*, To compare the activity of GAL4-VP16 with that of the glucocorticoid receptor (GR), CHO cells were co-transfected with the GAL4-VP16 effector plasmid (pSGVP) and the MMTV-CAT construct bearing either: no GAL4 binding site, pMC; UAS_G at -110, pMCU-110; or an MH100 GAL4 17 mer binding site⁷ at -110, pMC17-110. An effector plasmid expressing the glucocorticoid receptor (pRSVGR, ref. 6) was co-transfected with the wild-type MMTV-CAT construct (pMC) and cells were incubated in the absence (-Dex) or presence (+Dex) of 0.2 μ M dexamethasone as described previously⁶. As controls, CHO cells were transfected with plasmids bearing CAT genes fused to the SV40 early (SV40 CAT) or Rous sarcoma virus LTR (RSV CAT) vector.

Methods. 5 μ g each of the reporter and effector plasmids were transfected by the DEAE dextran technique as described by Cato *et al.*²¹. Forty-eight hours post-transfection, cells were collected and assayed for CAT activity as described⁶. GAL4 and derivative coding sequences were cloned from their respective parent plasmids into the pECE vector²⁷ using restriction fragments as follows: pSG4 and pSG147, EcoRI from pAG4 and pAG147 (ref. 6); pSGB17, HindIII/SalI from pB17 (ref. 2). To construct the GAL4-VP16 fusion a BglIII/end-filled HindIII fragment from pCRF2 (ref. 11) encoding the 78 carboxy-terminal amino acids of VP16 was cloned into a pB17 BamHI/end-filled SalI backbone. The resulting plasmid, pMA540, was used to express GAL4-VP16 in yeast (data not shown). The linker joining GAL4 and VP16 encodes the following peptide: Pro-Gly-Phe-Pro-Gly-Ile-Trp. pSGVP was constructed by subcloning the HindIII fragment encoding the fusion construct into pECE²³. The MMTV-CAT reporter construct (pMC) was made by subcloning the XbaI/BamHI fragment containing MMTV LTR-CAT from pGMCs (ref. 6) into p118t.2, which has a pUC118 backbone with a 360-bp transcriptional terminator²³ upstream of a multiple cloning site. The UAS_G was isolated from pMA552 (containing the 365-bp UAS_G (ref. 16) in the BamHI site of pUC18) as a SacI/KpnI fragment, made blunt with T4 polymerase and ligated into the SacI, XbaI, or BamHI sites (made blunt as appropriate) of pMC. pMC17-110 was constructed by subcloning a SmaI/HindIII fragment, containing a MH100 17 mer, of pMH100 (ref. 7) into the SacI site of pMC (both fragments made blunt).

wild-type GAL4 or GAL4-B17—the latter activator is GAL4 (1-147) fused to an acidic activating region encoded by an *Escherichia coli* genomic fragment². Figure 2 also shows that GAL4-VP16 worked, albeit about 10-fold less efficiently, when the UAS_G was positioned at -1180 or +1850. Using a different cell line (CHO-DUKX (ref. 6)), GAL4-VP16 worked at about the same level of efficiency from all three positions (Fig. 1c). Activity was not detected in either cell line for either GAL4 or GAL4-B17 when the UAS_G was positioned at -1180 or +1850 and in no case did GAL4 (1-147) activate, confirming previous results⁶. The start sites of the induced RNAs for all constructs were identical to those of the glucocorticoid-receptor-stimulated transcripts initiated from the wild-type MMTV LTR (not shown). Immunoprecipitation and western blotting of the various activator proteins revealed that all were produced at equal levels (within a factor of two or three) in both cell types (data not shown). In all of these experiments the reporter bears a binding site for the mammalian activator nuclear factor (NF1) near the TATA sequence, and we do not know what contributions, if any, this protein made to the activation we observed. It is possible, for example, that the differences observed between the two cell lines reflect different concentrations of this auxiliary factor.

GAL4-VP16 activated only slightly more efficiently from a UAS_G (which includes four GAL4 binding sites) than from a single strong binding site (17 mer, Fig. 1d), indicating that activation may be near-maximal from the single site. In addition, GAL4-VP16, bound at position -110, activates transcription about 10-fold more efficiently than the glucocorticoid receptor (GR) bound to the multiple sites found in the wild-type MMTV promoter (Fig. 1d). Finally, Fig. 1d shows that GAL4-VP16 bound at -110 elicits a level of CAT activity that is about that produced by a CAT gene driven by an adjacent simian virus 40 (SV40) or Rous sarcoma virus (RSV) promoter. For reasons we do not understand, using an SV40 promoter as a measure, we find that wild-type GAL4 is substantially less active than reported by Webster *et al.*⁷.

Why is GAL4-VP16 such a powerful activator? It is highly unlikely that the distinctive properties of the molecule can be ascribed to, for example, increased DNA binding. A variety of experiments suggest that GAL4 (1-147) forms stable dimers (Carey, M., Kakidani, H. and M.P., in preparation) and that this fragment, as well as other GAL4 derivatives, efficiently fill the GAL4 binding sites *in vivo*¹⁷. Instead, we imagine that the acidic region of VP16 interacts with unusual avidity with some component of the transcriptional apparatus, perhaps the TATA-binding factor TFIID (ref. 18). In other experiments, we found that GAL4-VP16, like native VP16, inhibited transcription initiating at promoters lacking GAL4 binding sites. As described elsewhere¹⁹, we believe this inhibitory effect (which we have called squelching^{19,20}) is an unavoidable consequence of expression of a strong activating region. If so, the continuous expression at high levels of a powerful activator would be harmful to cells, but such an activator could profitably be used by a virus that expressed it transiently²⁰.

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Structure of antibody hypervariable loops reproduced by a conformational search algorithm

Robert E. Bruccoleri*, Edgar Haber* & Jiří Novotný*

Cellular and Molecular Research Laboratory,
Massachusetts General Hospital & Harvard Medical School, Boston,
Massachusetts 02114, USA

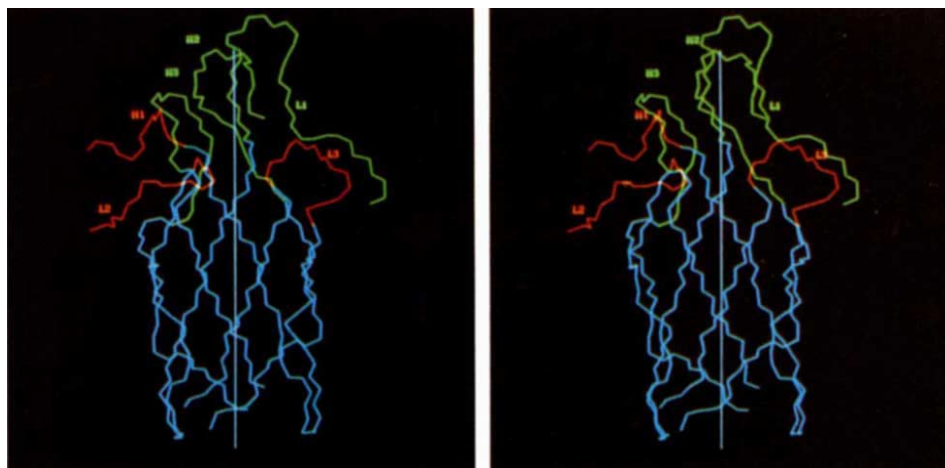
The antigen-combining site of antibody molecules consists of six separate loops supported by a conserved β -sheet framework; antibody specificity arises from length and sequence variation of these 'hypervariable' loops¹ and can be manipulated by transferring sets of loops between different frameworks². Irregular loops are the most difficult parts of protein structure to understand and to model correctly³⁻⁶. Here, we describe two computer experiments where all the hypervariable loops were deleted from X-ray structures of mouse immunoglobulins and reconstructed using the conformational search program CONGEN⁷. A protocol was developed for reconstruction of the hypervariable loops in McPC 603 antibody. Calculated loop conformations were generated and a model of the combining site was built from selected low-energy conformations. We then modelled hypervariable loops in another antibody molecule, HyHEL-5. Both models agreed well with the known crystal structures. Our results hold out promise for the success of future modelling studies of complete antigen-combining sites from amino acid sequences.

Current techniques of loop-structure modelling, and antibody-binding site modelling in particular⁸⁻¹³, involve a variety of methods. Protein conformation is determined by values of backbone and side-chain torsional angles ϕ (C-N-C α -C), ψ (N-C α -C-N) and χ (N-C α -C β -C γ , C α -C β -C γ -C δ and so on). Three-dimensional modelling involves finding specific values for all these torsional degrees of freedom. A particularly promising approach is therefore to use automatic computer algorithms that uniformly sample the complete conformational space of a polypeptide chain segment^{7,14-16}. Theoretically, all the backbone and side-chain conformations compatible with the rest of the protein structure can be generated. The lowest free-energy conformation should correspond to the naturally occurring one. In practice, technical problems associated with an exhaustive sampling of conformational space restrict searches to short polypeptide segments only. A successful protocol for hypervariable loop reconstruction could later be extended to automatic modelling of combining sites of unknown three-dimensional structures solely from their amino acid sequences.

We used X-ray atomic coordinates of the mouse immunoglobulin McPC 603 (ref. 17) as our starting structure, and defined endpoints of the hypervariable loops as shown in Fig. 1. These six loops, 46 amino acid residues altogether, were deleted from the structure. The shortest loop, H1, contained five

* Present address: The Squibb Institute for Medical Research, Princeton, New Jersey 08543-4000, USA

Fig. 1 Stereoscopic view of the hyper-variable loops of McPC 603 with the underlying β -sheets. By tight non-covalent association, two curved β -sheets from the VL and VH domains create a twisted, elliptical β -barrel^{3,21,22}. The polypeptide backbone of this barrel can be approximated by mathematical hyperboloidal surfaces²² and the long axis of the least-squares fitted surface becomes a convenient reference axis of the β -barrel. The fitted axis is drawn as a white vertical line, the β -sheet backbones are drawn in blue lines (the VL domain sheet in front, the VH domain sheet behind and the six hypervariable loops (heavy-chain loops H1, residues 28–32; H2, residues 50–58; H3, residues 102–109; light-chain loops L1, residues 26–37; L2, residues 56–61; L3, residues 97–102; consecutive numbering system) are in red and green. The three shorter loops, H1, L2 and L3, drawn in red, have lower average axis coordinates, so they are closer to the centre of the barrel than the other three loops, H2, H3 and L1, drawn in green. In our construction protocol, the low loops were constructed before the high loops.



residues; the longest, L1, contained 12 (see Fig. 1 legend for loop nomenclature).

The CONGEN search algorithm⁷ uses a selectable angular grid to sample the torsional degrees of freedom in a given polypeptide segment, using the amino- and carboxy-terminals as fixed endpoints. In the searches, both *cis* and *trans* proline peptide bonds are considered. Each sampling run generates a set of loop conformations which satisfy the fixed endpoint condition (the modified Gō and Scheraga chain-closure algorithm)^{18,19} and have acceptable potential energies²⁰. These energies are used to distinguish between 'good' and 'bad' conformations and depend critically on short-range interactions between loops and, consequently, on the order of loop construction. Our order of loop reconstruction L2-H1-L3-H2-H3-L1 was

chosen on the basis of the relative positions of the loops in the local frame of reference provided by the β -sheets of the VL-VH domain interface^{21,22} (Fig. 1). The tight non-covalent interaction of these sheets creates an elliptical ' β -barrel' whose long axis nearly coincides with the two fold axis of VL-VH domain pseudosymmetry. In this reference frame, the three shorter loops (L2, L3 and H1) are placed lower (closer to the midpoint of the β -barrel interface) than the others. They do not interact with each other and provide a natural basis for the construction of the remaining 'high' loops, H2, H3 and L1. These 'low' loops were built first, H2 next, and loops H3 and L1, which interact closely, were built last.

From the set of conformations generated for each loop, 'the best one' was selected to be incorporated into the final model

Table 1 Description of McPC 603 combining site reconstruction

Loop	Run and conformation number	Total conformations found	Energy (kcal)	Root mean square (Å)		Surface (Å ²)
				Total	Backbone	
L2	56LMCP7-75*	159	-14.6	1.9	1.6	360
	56LMCP7-89		-16.1	2.4	2.0	397
H1	28HMCP1-44*	46	-17.1	1.7	0.7	344
	28HMCP1-43		-10.7	1.6	0.7	329
L3	97LMCP7-42*	306	-20.8	1.4	0.8	320
	97LMCP7-95		-21.8	3.0	1.5	375
H2	50HMCP5-81	576	-18.8	2.1	2.0	101
	50HMCP5-75		-18.5	2.2	2.2	126
	50HMCP5-71		-18.2	2.1	2.1	100
	50HMCP5-76		-17.9	2.2	2.1	123
	50HMCP5-170*		-17.8	1.6	1.0	80
	50HMCP5-175		-17.8	1.7	1.0	96
	50HMCP27-681*		-39.6	2.4	1.9	550
	50HMCP27-874		-33.8	4.0	1.5	584
	102HMCP17-176*		-64.5	2.9	1.1	369
	102HMCP17-408		-62.2	2.7	0.9	394
L1	26LMCP23-1344*	2,287	-49.0	4.0	3.5	799
	26LMCP23-1348		-46.9	4.4	3.8	810

Six polypeptide chain segments (see Fig. 1) were deleted from the McPC 603 structure and were reconstructed in separate computer runs, in the order listed in the table. In the L2 loop, residues 61–59 were searched in the C $\rightarrow$ N direction and the Gō-Scheraga chain-closing algorithm^{7,19} was applied to residues 56–58. In the H1 loop residues 28 and 32 were searched and the chain-closing was applied to residues 29–31. In the L3 loop, residues 97–99 were searched successively and chain-closing applied to residues 100–102. In the H3 loop the residues were searched successively from the amino-terminus and the chain-closing routine was applied to residues 107–109. The H2 and L1 loops were too long to be constructed as one segment and 'real space renormalization'³⁰ was applied. In H2, the N-terminal tripeptide Ala-Ser-Arg was searched first (see the top six conformations listed in the table). From the six lowest-energy conformations, all within a 1 kcal interval, the one with the smallest surface was taken as the starting conformation for the search over the rest of the loop (see the lower two conformations listed in the table). In the L1 loop, three separate runs constructed dipeptides Ser-Gln, Ser-Leu and Leu-Asn. In the second and third runs, the four lowest-energy conformations from the previous run were all included as starting conformations. The C-terminal dipeptide Lys-Asn was then constructed separately. Finally, the lowest-energy conformations of the peptides Ser-Glu-Ser-Leu-Leu-Asn (residues 26–31) and Lys-Asn (residues 36–37) were included as starting conformations into the search over the rest of the loop. The angular grid on which the torsional spaces were searched was 30° and the non-bonded interactions were computed within the cut-off distance of 5 Å, with dielectric constant, $\epsilon = 50$ (the three 'low' loops of Fig. 1), or using cut-off distance of 8 Å, with distance-dependent dielectric constant (the three 'high' loops of Fig. 1). The lowest conformations of each run are given; *, indicates those included in the model in Fig. 2.

Table 2 Description of HyHEL-5 combining site reconstruction

Loop	Run and conformation number	Total conformations found	Energy (kcal)	Root mean square (Å)		Surface (Å ²)
				Total	Backbone	
L2	49LHYHEL55-76*	100	-57.6	1.7	0.8	268
	49LHYHEL55-89		-56.3	2.3	1.3	294
	49LHYHEL55-20		-55.2	1.9	1.1	286
H1	28HHYHEL55-21*	37	-26.1	1.8	1.1	489
	28HHYHEL55-22		-22.9	1.9	1.2	498
L3	90LHYHEL56-21*	33	-20.4	4.1	1.1	357
	90LHYHEL56-18		-5.5	4.4	1.4	368
H2	50HHYHEL56-4*	6	-0.3	3.1	2.1	205
	50HHYHEL56-3		2.5	3.2	2.1	214
H3	100HHYHEL55-11*	14	-47.3	2.7	1.0	359
	100HHYHEL55-2		-46.7	1.5	0.9	381
L1	26LHYHEL55-11	13	-35.1	2.0	0.4	367
	26LHYHEL55-7*		-32.8	1.8	0.6	362

Amino acid sequences of variable domains of McPC 603 and HyHEL-5 light and heavy chains were aligned to maximize sequence homology (43% identical residues in the VH domain and 54% identical residues in the VL domain). Endpoints of HyHEL-5 hypervariable loops were then determined as residue positions coinciding with the endpoints of McPC 603 hypervariable loops determined previously (see Fig. 3 legend). The six HyHEL-5 hypervariable loops defined in this way (32 residues altogether) were deleted from the HyHEL-5 crystallographic structure and reconstructed using the protocol developed for construction of the McPC 603 combining site. The order of loop construction, Gö-Scheraga chain-closure, and so on was as described in Table 1. More specifically, the L2 loop residues 54-52 were searched in the C → N direction and the chain-closing algorithm^{7,19} was applied to residues 49-51. In the H1 loop the residues 28 and 32 were searched and chain-closing applied to residues 29-31. In the L3 loop, the residues 90 and 91 were searched successively and chain-closing applied to residues 92-94. In the H2 loop the N-terminal tetrapeptide 50-53 was searched first and the chain-closing was applied to residues 54-56. In the H3 loop residue 100 was searched and chain-closing applied to residues 101-103. In L1, the residues 26 and 30 were searched and chain-closing applied to residues 27-29. Because of the short lengths of the HyHEL-5 loops H2 and L1, whole loops could be constructed in single search runs. The angular grid on which the torsional spaces were searched was 30° and the non-bonded interactions were computed using cut-off distance of 8 Å, with the distance-dependent dielectric constant used for all six loops. The lowest-energy conformations of each run are given; * indicates those included in the model displayed in Fig. 3.

of McPC 603 as follows. All the conformations were ranked by their empirical potential energies²⁰; the two lowest-energy conformations were examined in detail. If calculated energies of these two structures differed by about 2 kcal (three times the Boltzmann factor kT , indicating that both conformations are well populated at room temperature), the one with the smaller solvent-exposed surface²³ was selected. Otherwise, the lowest-energy structure was selected. The H2 and L1 loops were too long to be constructed in a single computer run. Instead, selected results of searches over shorter sections of the loop were included into new searches over the rest of the loop as starting conformations (see Table 1). The success of our reconstruction was gauged against the original X-ray structure, by evaluating root-mean-square (r.m.s.) deviations between the computed and the crystal conformations.

Details of individual loop constructions in McPC 603 are described in Tables 1 and 3. Overall, the best r.m.s. conformations were not often the lowest energy ones and therefore could not be selected in a true modelling experiment where the final structure would be unknown. Nevertheless, there were low-energy conformations generated which still matched the native structure fairly well. The r.m.s. agreement of our model, constructed from the few selected lowest-energy conformations, is 2.4 Å total and 1.7 Å backbone r.m.s. shifts from the X-ray structure (Fig. 2). The model shows close agreement with the crystal; for example, the rare *cis* peptide bond conformation of Pro L101 was accurately reproduced. Two isolated regions show large disagreements with the crystal structure: the tyrosine side chain ring H103, which had been misplaced by rotation of about 160° around its χ_1 torsional angle (Fig. 2d) and the backbone of the middle of the L1 loop, residues 32-35 (Fig. 2b).

Each of the six McPC 603 loops was very closely matched by at least one of the computed conformations. If these 'best' r.m.s. constructions could be assembled into a model, its shift from the X-ray structure would be 1.7 Å, 1.3 Å on the backbone only. This level of accuracy approaches the limits of atomic resolution of the McPC 603 structure¹⁷ (0.5-1.0 Å).

The reconstruction protocol developed for McPC 603 hypervariable loops was next applied to another antibody structure, HyHEL-5, to check its generality. The six HyHEL-5 hypervariable loops (32 residues altogether) were reconstructed essentially

as described for the McPC 603 loops; the only change to the above construction protocol was a consistent use of the distance-dependent dielectric constant, $\epsilon = R$ (R is distance between the two interacting charges), in the evaluation of electrostatic interactions. As for McPC 603, each of the hypervariable loops was matched closely by at least one calculated conformation. The model of the HyHEL-5 combining site, made from selected lowest-energy conformations showed a 2.6 Å r.m.s. shift from the X-ray structure, 1.4 Å r.m.s. from the backbone. Thus, our construction scheme developed on the McPC 603 molecule could be used, with only small technical modifications, on a different molecule to give an equally good result.

Figure 3 shows details of our HyHEL-5 model. Loop backbones show good agreement with the X-ray structure, except for the H2 loop residues H51-H54. Most of the side chains match the X-ray structure equally well, the exceptions being Tyr H101 and Arg L92. The poor agreement in the H2 modelling is due to inaccurate positioning of the side chain Phe H29 during the H1 loop construction. The shift in the Phe ring position,

Table 3 CONGEN reconstruction of combining sites in antibodies McPC 603 and HyHEL-5

Loop	Length	Root mean square to crystal (Å)		CPU time*
		total	backbone	
H1 in McPC 603	5	1.7	0.7	4 hours
H1 in HyHEL-5	5	1.8	1.1	3 hours
H2 in McPC 603	9	2.1	1.6	5 days
H2 in HyHEL-5	7	3.1	2.1	20 minutes
H3 in McPC 603	8	2.9	1.1	7 days
H3 in HyHEL-5	4	2.7	1.0	2 hours
L1 in McPC 603	12	3.0	2.6	7 days
L1 in HyHEL-5	5	1.8	0.6	40 minutes
L2 in McPC 603	6	1.9	1.6	8 hours
L2 in HyHEL-5	6	1.7	0.8	37 hours
L3 in McPC 603	6	1.4	0.8	5 hours
L3 in HyHEL-5	5	4.1	1.1	12 hours
All loops, McPC 603	46	2.4	1.7	20 days
All loops, HyHEL-5	32	2.6	1.4	2.25 days

* Time on central processing unit, a MicroVax II.

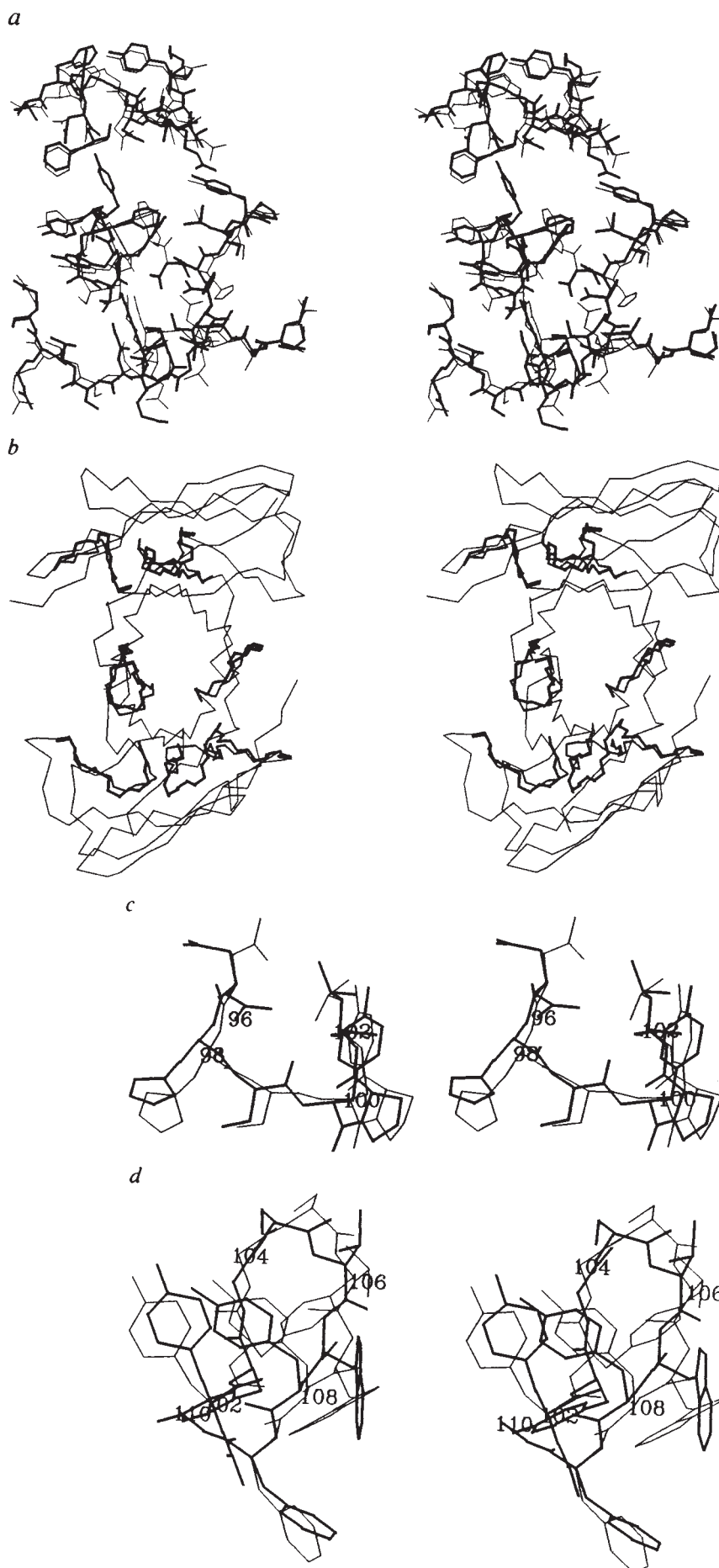


Fig. 2 Stereoscopic view of the McPC 603 antigen combining site, as reconstructed by CONGEN (see Table 1). Light lines trace the crystallographic structure, heavy lines are CONGEN-generated conformations. *a*, Comparison of all six hypervariable loops in the X-ray structure and those constructed by CONGEN. The loops are clockwise from the top: H2, H-chain residues 50–58 (Ala-Ser-Arg-Asn-Lys-Gly-Asn-Lys-Tyr); L3, L-chain residues 97–102 (Asp-His-Ser-Tyr-Pro-Leu); L1, L-chain residues 26–37 (Ser-Gln-Ser-Leu-Leu-Asn-Ser-Gly-Asn-Gln-Lys-Asn); L2, L-chain residues 56–61 (Gly-Ala-Ser-Thr-Arg-Glu); H3, H-chain residues 102–109 (Tyr-Tyr-Gly-Ser-Thr-Trp-Tyr-Phe); and H1, H-chain residues 28–32 (Thr-Phe-Ser-Asp-Phe). *b*, Backbones of the framework residues outside the hypervariable loops are traced through α -carbons only, hypervariable loop backbones are drawn as N, C α and C atoms. Orientation of the combining site was as above. The middle part of the L1 loop, where there is the greatest discrepancy between the crystallographic and calculated structures, is poorly visible and disordered in the crystal¹⁷. *c*, A detailed picture of the L3 loop (total r.m.s. shift between the crystallographic and calculated conformations 1.4 Å, backbone shift 0.8 Å). Note the *cis* proline residue L101. *d*, A detailed picture of the H3 loop (total r.m.s. shift between the crystallographic and calculated conformations 2.9 Å, backbone shift 1.1 Å). Note the displacements of the side-chain ring Tyr H103 and, to a lesser extent, Trp H107. These inaccuracies are largely responsible for the unsatisfactory r.m.s. value.

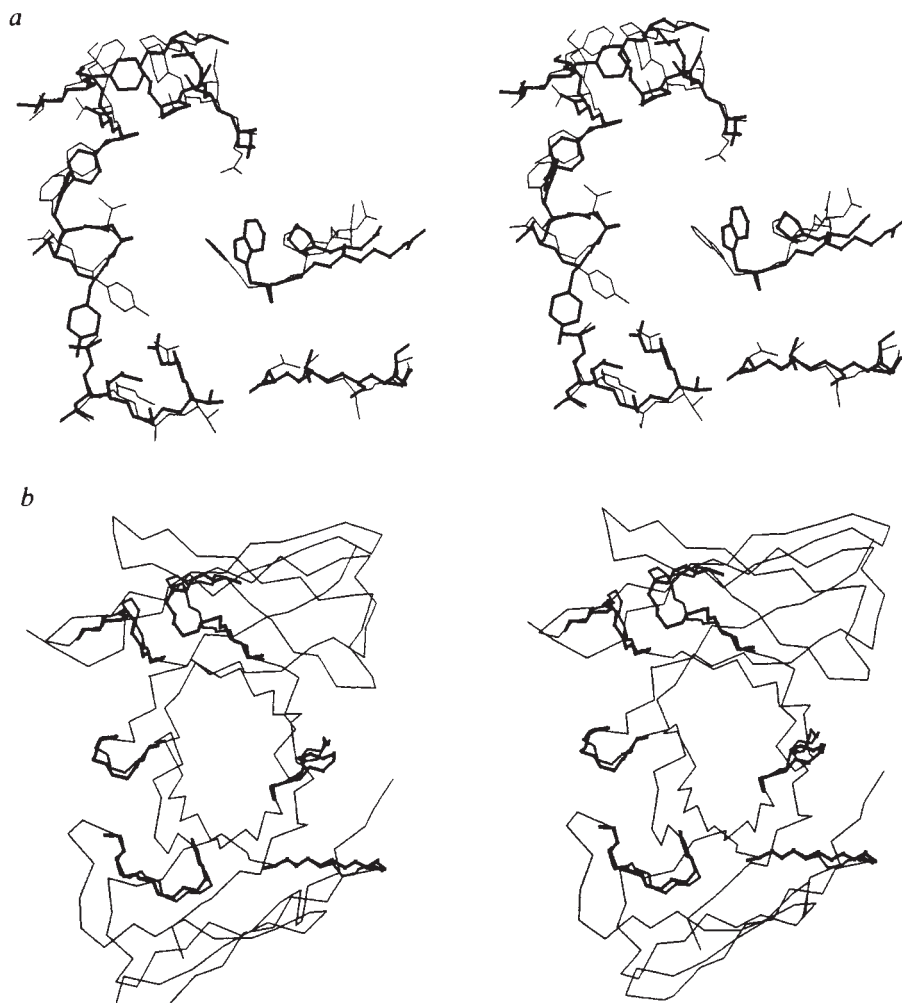


Fig. 3 Stereoscopic view of the HyHEL-5 antigen combining site, as reconstructed by CONGEN (see Table 2). Light lines trace the crystallographic structure, heavy lines are CONGEN-generated conformations. *a*, Comparison of all six hypervariable loops in the X-ray structure and as constructed by CONGEN. The loops are, clockwise from the top: H2, H-chain residues 50–56 (Glu-Ile-Leu-Pro-Gly-Ser-Gly); L3, L-chain residues 90–94 (Trp-Gly-Arg-Asn-Pro); L1, L-chain residues 26–30 (Ser-Ser-Ser-Val-Asn); L2, L-chain residues 49–54 (Asp-Thr-Ser-Lys-Leu-Ala); H3, H-chain residues 100–103 (Asp-Tyr-Asp-Phe); and H1, H-chain residues 28–32 (Thr-Phe-Ser-Asp-Tyr). *b*, Backbones of the framework residues outside the hypervariable loops are traced through α -carbons only, hypervariable loop backbones are drawn as N, C α and C atoms. Orientation of the combining site is as above.

although small, interfered with the correct placement of Pro H54 because of atomic volume overlaps and resulted in misplacement of the middle part of this loop. Discrepancies like these provide us with important stimuli for future work. We expect that improvements in energy ranking (for example, a better representation of solvent-modified electrostatic interactions) will allow selection of better-quality conformations and construction of more accurate models. Empirical free-energy functions^{24–27} should be particularly useful. Likewise, canonical structures developed by Chothia and Lesk¹³, could be used to improve on the loop selection process.

The conceptual basis for the construction of polypeptide loops on a fixed backbone framework is only beginning to be understood. *A priori*, there was no guarantee that local potential energies of the loops and their local environments would lead to accurate models of the structure. The success of the construction protocol may imply that local interactions in protein structure²⁸, particularly loops²⁹, are more important than generally believed.

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Designing and planning a laboratory

S. McKenney

A laboratory's typical tasks, required instruments, number of staff, and the infrastructure of the building in which it is housed must all be considered in its planning and design.

FREQUENTLY, the operating budget of a small laboratory does not allow for the expense of hiring a consulting architect or engineer to plan a new laboratory facility or to renovate an old one. The design and implementation of the project often becomes a joint project between the laboratory supervisor or facilities manager and the laboratory employees. The ultimate objective is to arrange for an efficient compromise between the need for work and storage space and the costs for that space. The following discussion should help in the evaluation of laboratory needs, and in the design of an efficient laboratory for any particular application.

Physical layout

Whether one is renovating an existing laboratory or moving into new laboratory space, it is essential to be familiar with the laboratory's physical environment. Among the many details which must be noted or taken into consideration during the planning stage are the wall-to-wall dimensions, or the physical boundaries of the laboratory, and the ceiling height and type. The second is critical when considering the installation of oversized equipment, such as fume hoods.

The location of drains, and power and utility lines must be discovered to enable the placement of sinks, and electrical and service outlets, and the location and dimensions of any columns or beams which may restrict the installation of casework must be mapped out. Enough room must also be left for existing floor models or oversized equipment such as refrigerators, ovens, incubators and autoclaves, keeping in mind requirements for maintenance access or storage space.

Fume hood location

If the laboratory requires a fume hood, it is very important that it be located correctly. The fume hood is the most important piece of safety equipment in any laboratory. For the hood to operate safely and efficiently, care must be taken in its placement.

There are essentially two parameters to take into consideration during the planning of fume hood placement: the design of the ductwork system, and the location of the hood within the laboratory. The ductwork provides for the containment of toxic or noxious fumes during their passage from the hood to the outside atmosphere. It is recommended that the blower be placed at the far end of the duct,

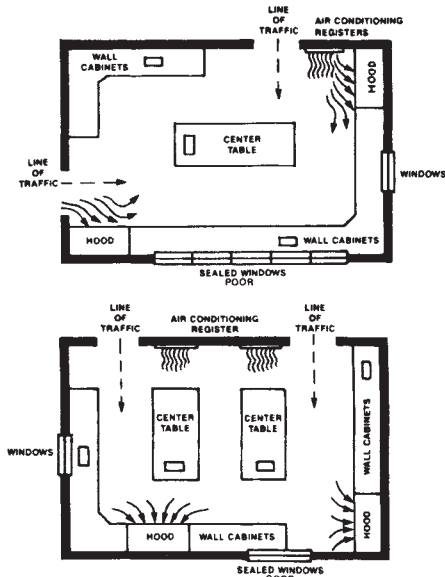


Fig. 1 Comparison of laboratories with poor (top) and optimal (bottom) hood placement.

remote from the fume hood itself, to create a negative pressure within the ductwork and reduce the noise of the blower. A negative pressure within the duct system will contain the fumes within the duct, but locating a blower within or near the hood generates a positive pressure in the duct which will tend to force fumes out of any leaks and into the laboratory or building atmosphere.

The ducting system should also be designed to be as short as possible, with few twists and turns. A short, straight duct system will allow the use of a smaller, less expensive motor/blower. Consult a local heating vent and air conditioning engineer for advice on the local codes regulating duct material and exhaust policies. A professional should also be consulted properly to size the motor/blower for the particular application required.

A fume hood operating at 100 f.p.m. is moving air at slightly more than 1 m.p.h. A person walking by the hood at 3 m.p.h. will create enough turbulence to disrupt air flow to the hood, and even to pull fumes from within the hood. Similar problems also occur if the hood is placed too close to windows, open doors, room air inlets or exhausts. Figure 1 compares a laboratory with poorly located fume hoods with one where the hoods have been placed correctly. Whenever possible, attempt to place the hood in the most efficient location, and build the rest of the laboratory around the hood.

Space requirements

The most common error made in the design of new laboratories is not allowing sufficient space. Each laboratory technician requires eleven linear feet of bench space – in addition to space dedicated to hoods, sinks and other instrumentation – and roughly 100 square feet of space overall. The aisle space should not be smaller than or significantly larger than four feet.

In order to design the best workflow into a new laboratory, it is important to understand what type of work is to be done in the laboratory. Also consider whether or not the type of work or the methods are likely to change over time.

Most real-life applications fall somewhere on a continuum between tightly compartmentalized and totally open designs. Compartmentalization is appropriate for research projects conducted by a single individual or for complete processes which require isolated floor space. The open design may be desirable where total flexibility is required, such as in a quality control laboratory, where an incoming sample may take one of several different paths.

Figure 2 illustrates floor plans and designs for both compartmentalized and open laboratories. The particular work patterns and methods used by a laboratory will dictate the best design for that labora-

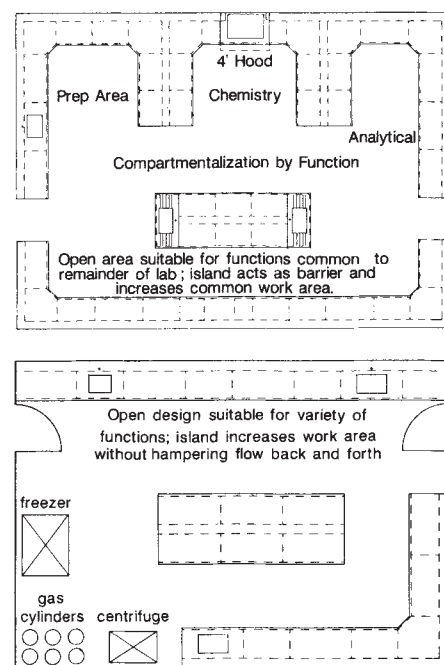


Fig. 2 Laboratory designs according to workflow.

tory: an instrumentation laboratory has very different requirements from those of a wet chemistry laboratory.

Additional considerations to keep in mind when planning a laboratory are:

Depth and type of worktops. Is chemical resistance a priority? Are there large instruments which will require extra bench space? Can bench space be maximized by using undercounter equipment?

Drawer and door configuration of base cabinets. Will cupboard space be needed for bulk storage, or are drawers more necessary?

Wall-mounted storage. Will storage shelves above benches be required?

Safety measures. Should safety showers, eyewash stations, fire blankets, chemical spill kits, fire extinguishers and first aid kits be installed? What are the laboratory requirements for sit-down (apron) areas?

Implementation

After the ideas for a new laboratory have been organized, it is a good idea to consult with casework dealers. Most offer furniture specialists and support groups, and can assist with blueprints and technical information.

When the time schedule for completion of the laboratory renovations is established, it must be kept in mind that there are any number of situations which may arise to slow down a project, many of which are beyond control. Enough time should be allowed so that if there are any surprises, they will not become major frustrations.

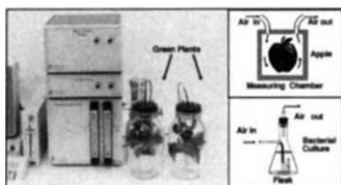
A new laboratory is an investment that should last for years. If the investment is made in a quality product which is installed correctly, it should profit the laboratory and its employees for years to come. □

Steven McKenney is at VWR Scientific, 3041 Skyway Circle North, Irving, Texas 75038, USA. For more information, fill in reader service number 100.

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Labweek roundup

At the Labweek exhibition next week in London, UK scientific instrumentation manufacturers will be showing off their latest wares. Among the new products to be launched are an FTIR spectrometer, a refractometry-viscometry detector and an analyser for enzyme immunoassays.

ALL of the products on display in Millipore's booth will no doubt be included in its new general **catalogue**, released this summer (*Reader Service No. 101*). The free comprehensive publication contains more than 300 pages of application and technical information on Millipore's products for the analysis, purification, monitoring and quality control of liquids and gases. Also included is information on the range of separations technology products offered by the company, from microporous membrane filtration, ultra-filtration, and ion exchange separation equipment, to reverse osmosis instruments.

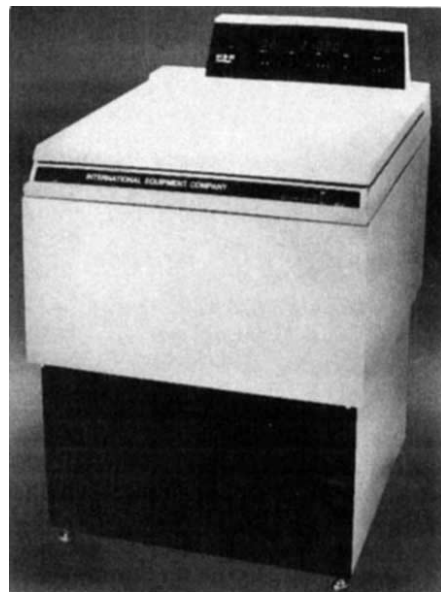
For breaking up research samples, MSE Scientific Instruments has developed the Soniprep 150 **ultrasonic disintegrator** (*Reader Service No. 102*). The Soniprep 150 is encased in a sound-proofed material to ensure that its ultrasound output is contained within the instrument, but the sample can be viewed throughout the operation through the Soniprep 150's transparent door. Automatic feedback control of the 23 KHz generator allows optimal excitation of the piezo-electric transducer to be maintained consistently, and the Soniprep 150's precision ampli-



MSE Scientific's disintegrator uses ultrasound.

tude meter affords good reproducibility of sonication conditions, says the company. The £2,850 (UK) standard unit comes with a 10-minute timer. For programming alternating sonication/cooling cycles, a £425 (UK) process timer unit can be added on.

Damon/IEC (UK) Ltd will be launching its new model B-22 high-speed **floor-standing centrifuge** at Labweek (*Reader*



Damon/IEC's floor-standing centrifuge allows speed to be tracked during the run.

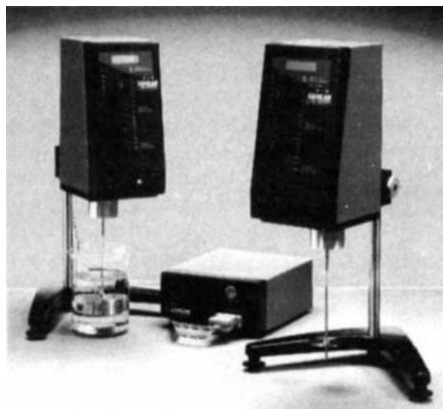
Service No. 103). The £10,600 (UK) centrifuge displays both set and actual conditions continuously over the duration of the run. Damon/IEC says the centrifuge is accurate to $\pm 1^\circ\text{C}$ from -9°C to $+39^\circ\text{C}$, and to within 20 r.p.m., up to the maximum speed of 22,000 r.p.m. ($49,300 \times g$). The model B-22 can hold up to 3,000 ml of sample, and has been designed with rapid acceleration and deceleration programs so that separations can be made in as little as 12 minutes. A range of 20 different rotor types has been developed for the model B-22, and each comes with a seven-year guarantee.

Thick and thin

Roth Scientific will be exhibiting its model 200 combined **differential refractometry-viscometry detector** for the first time in the United Kingdom at this year's Labweek (*Reader Service No. 104*). The £20,000 (UK) instrument is manufactured jointly by Viscotek in the United States and Knauer in West Germany, as a component to be used in gel permeation chromatography. The detector allows the construction of a universal calibration curve from any convenient polymer standard, says the company. The curve can then be used to determine the molecular

weight distribution of unknown polymers, whether they be linear, branched, homopolymeric or copolymeric. Data required for laser light scattering techniques, such as refractive index and second virial coefficient, are not necessary. The detector may also be used to obtain intrinsic velocity, Mark-Houwink constants, branching frequency and radius of gyration.

A new range of microprocessor-driven **rotational viscometers** has recently been introduced by Camlab Ltd (*Reader Service No. 105*). Camlab says the viscometers use proven viscometric techniques in conjunction with a specially developed precision angular torque transducer, to provide accuracy to within 1 per cent of the full scale. The instruments measure viscosity continuously, and display the results in either per cent full scale or viscosity units of centipoise, poise or kilopoise. Operating over sixteen crystal-

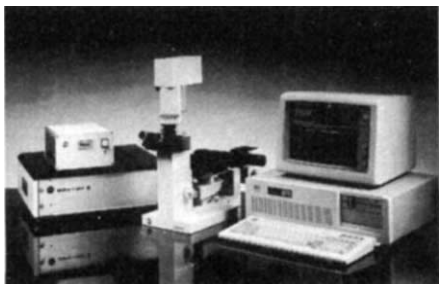


Two examples from the Camlab Ltd range of rotational viscometers.

controlled speeds, the viscometers also have an auto zero facility, display hold, spindle selection, overrange and under-range indicators, and a recorder output for logging data. Four models are available; prices start at £1,695 (UK).

Microplate matters

A computer-based microscope for the analysis of **antigen-antibody reactions** in routine clinical work is new from E. Leitz Instruments (*Reader Service No. 106*). The £28,000–30,000 (UK) Patimed photo-metrically records the reactions in Terasaki-type microtitre trays, and can be



Speedy analysis of antigen-antibody reactions is possible with the Leitz Patimed.

used in HLA testing and techniques to differentiate between live and lysed cells. Leitz says the system can screen, measure and record results from a 60-well tray in less than 30 seconds. The software package provided with the instrument includes programs for automatic HLA phenotyping and antibody screening analysis.

Labsystems will be exhibiting a wide range of microplate-based equipment at Labweek, and will be showing for the first



Labsystems' answer for the automatic analysis of enzyme immunoassays: the Auto-EIA.

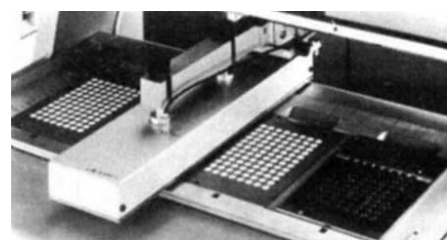
time in the United Kingdom an **automatic analyser for enzyme immunoassays**, named the Auto-EIA (*Reader Service No. 107*). The £30,000 (UK) Auto-EIA automatically controls sample dispensing, incubation, washing and data handling, through assay protocols stored in the computer's memory. The instrument's random access profiling feature allows it to run up to 12 different assays on each sample.

Berthold Instruments has recently introduced to the market an **automated harvester-analyser** sample handling sys-



First the harvester harvests all 96 wells . . .

tem designed for procedures involving the collection of radioactive precipitates or the harvesting of cells on filters for subsequent radionuclide analysis (*Reader*



. . . then the radioactivity detector counts the filter using an automatic detector head.

Service No. 108). The system consists of a harvester capable of processing all 96 wells of a microtitre plate in one step and a radioactivity detector which permits the direct quantitative analysis of filters. The contents of a microtitre plate are simultaneously collected on a filter — a process that the company says takes less than two minutes — and automatically counted in the filter plate holder of the detector by a detector head under computer control. Simultaneous processing of all wells eliminates well-to-well contamination, says Berthold Instruments, and the elimination of the need for scintillation cocktails makes counting safer. Thirty protocols are built into the instrument's microprocessor.

Sample prep-arators

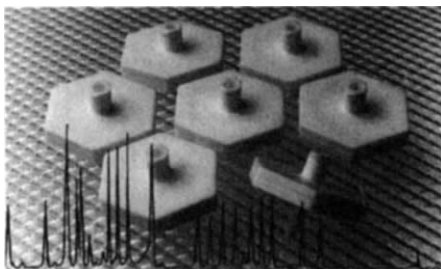
Gelman Science's display of **disposable filtration equipment** is sure to include its



The Gelman Science AcroPrep syringes can be used alone, or with the batch processor unit.

AcroPrep syringe-less small volume filtration unit (*Reader Service No. 109*). The AcroPrep combines a filter, a syringe and a filtrate vial into one unit. The filter plunger is hollow, and can serve as a collection vial for the filter sample, and every unit has a built-in pre-filter to prevent clogging. The AcroPrep filtration units are available with either PTFE or nylon membranes, with a pore size of 0.45 µm. No glues or adhesives are used in the manufacturing process, says the company, to reduce the level of extractables. The AcroPrep units can process up to 3 ml of liquid, and come 50 to a £57 (UK) box. For multiple sample filtering, the AcroPrep Processor, which can compress up to 40 AcroPrep units in 2 minutes, can be purchased for £850 (UK).

Anotec says its **inorganic membrane syringe filters**, dubbed Anotops, are ideal for both sample preparation and cold

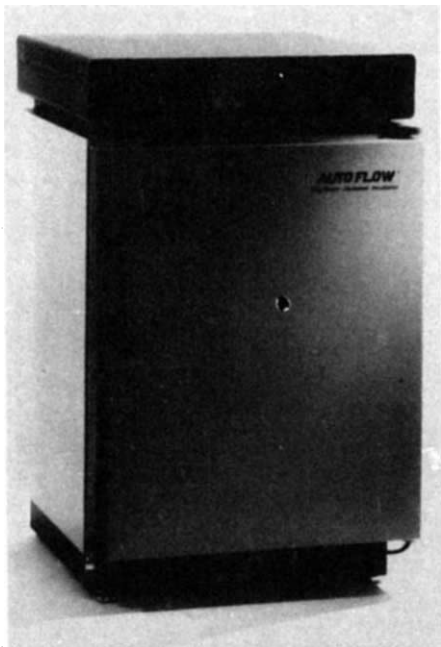


The Anotop inorganic membrane syringe filters can be used for sample prep and sterilization.

sterilization (Reader Service No. 110). The filters are available sterile or non-sterile in 25 mm and 10 mm sizes, with either 0.2 μm or 0.02 μm pores. The Anotops are compatible with a wide range of solvents, including concentrated nitric acid, glacial acetic acid, acetone and hexane, and its high porosity and thin profile provide high flow rates, says Anotec. The company also says the narrow size distribution range of the membrane contained within the Anotops ensures well-defined retention characteristics. The Anotops are supplied in boxes of 10 for £8–15 (UK) each.

Growth products

At the heart of the newest CO_2 incubator from Arnold R. Horwell Ltd is a new form



The CO_2 incubator from Arnold R. Horwell Ltd has technology born in the auto industry.

of CO_2 control which owes its origins to exhaust emission control systems developed in the automotive industry (Reader Service No. 111). The NuAire IR Auto-flow CO_2 incubators have infrared detectors which are sensitive to changing levels of CO_2 , but insensitive to changes in relative humidity, says Horwell. The shell of the NuAire incubators is manufactured from stainless steel, and is guaranteed by Horwell for five years. The incubators are water-jacketed to keep the temperature

within the work chamber as uniform as possible, and the outer door is insulated and heated to avoid condensation on the inner glass door. Horwell says the temperature variation in its 188-litre incubator is $\pm 0.2^\circ\text{C}$.

For bulk cell culture, Sterilin offers its Spira-Cel roller bottle (Reader Service No. 112). Available in three sizes, the Spira-Cel bottle consists of an inner polystyrene spiral which has been treated for cell attachment and growth, and an optically clear outer container. Sterilin says the



See-through culture capability with Sterilin's Spira-Cel roller bottles.

Spira-Cel provides greater culture area than conventional roller bottles, resulting in higher cell concentration and cell product yields. Both units are disposable, and the container has a special baffle tip to prevent spiral movement or liquid surge when emptying. The Spira-Cel has graduations from 50 to 1,000 ml along the side, a frosted area for identification marking, and a conical bottom to ensure complete removal of supernatant. Traction rings in opposing directions are provided to minimize bottle slippage. The Spira-Cel bottles come 20 to a box, and are wrapped in polystyrene.

The model B5061 Auto-Zero cell-culture incubator from Heraeus Equipment Ltd is an electrically controlled CO_2 incubator that incorporates a self-adjusting measuring system to keep the operating parameters constant and reproducible (Reader Service No. 113). The company says this feature makes manual recalibration of the CO_2 zero unnecessary, and is particularly useful when the incubator is used for long-term cultivation. A third acrylic door subdivided into six smaller doors is available as an option, to allow sections of the inner chamber to be reached without disturbing the environment of the other sections. The plug-in CO_2 thermal conductivity detector and the non-tilt shelves are removable for cleaning and disinfecting the inner casing, made of bactericidal copper or stainless steel.

The inner chamber can also be fitted with a UV sterilizer for further disinfection. The B5061 incubator costs £4,995–7,485.

Cherwell Laboratories has a media preparation sterilizer, the model FIP Agarster, which has an alpha-numeric



For large agar needs, Cherwell Labs' Agarster can hold up to 10 litres of media.

display with a membrane keypad for data entry (Reader Service No. 114). The device holds between 2 and 10 litres of agar, and has a special magnetic stirring system developed by Cherwell. The £8,900 (UK) Agarster's memory can store sterilization protocols, and a dot matrix printer can be used along with the instrument to record data on date, media reference and parameters on the chart record, and to generate a trace of the preparation cycle.

The small world

Nikon's Microphot-FXA fully automatic photomicroscope was designed with the non-microscopist in mind (Reader Service No. 115). The £20,000–25,000 (UK) microscope automatically calculates the correct scale value to measure the size of the specimen, and the motorized condenser



Nikon's Microphot-FXA photomicroscope can be fitted out with up to three cameras.

automatically sets the aperture according to the objective being used. All essential operations — from focusing to taking the final photomicrograph — can be performed with one hand, while observing the specimen. The motorized revolving nose-piece permits objectives to be changed quickly without interfering with observation, says Nikon. The $\times 1$ and $\times 20$ objectives also focus automatically to reduce errors in photomicrography at low magnifications. The FXA is provided with an RS232 port, enabling the microscope to be controlled by a PC or printer. It can be fitted with up to three cameras: two on the side, and one on top.

Olympus Optical will be introducing three new options to its CUE line of **image analysis packages** at the Labweek show (Reader Service No. 116). The CUE-2 system is now available with a high performance frame grabber and more powerful software, according to the company, and the CUE-3 has been updated to offer an additional facility for real colour analysis. The CUE-4 top-of-the-range system has a new inter-image function which allows up to four images to be stored and compared simultaneously. The CUE-4 can be operated in real time, and a large library of linear and non-linear filters is enhanced by user-defined filters in 3×3 , 5×5 , and 7×7 arrays. Fourier transforms, advanced statistics and menu-controlled user programming are also provided in the CUE-4 system.

Bits and bytes

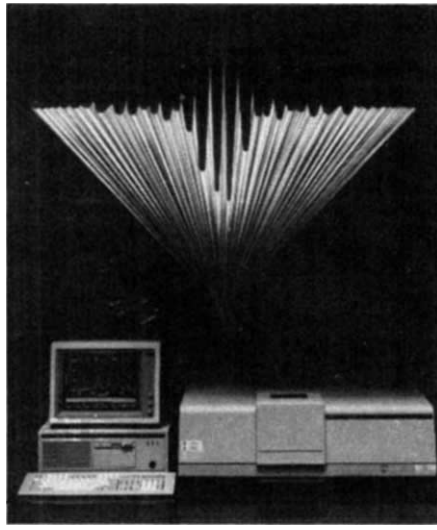
The Peak Master **chromatography data station** is new from Harley Systems, and is certain to make a showing at Labweek (Reader Service No. 117). The Peak Master has 20-bit resolution, a feature that Harley says allows a wide variety of peak heights and widths to be distinguished easily, and one that it says will offer advantages when analysing impurities, trace quantities and widely differing chemical species. Peak Master has four digital inputs and outputs per channel, enabling it to control other laboratory equipment such as autosamplers, valve changers, fraction collectors and chemistry process controllers. A timed integration events function helps users set the baseline and perform multiple peak integrations. Harley says the Peak Master can process a sampling rate of 100 per second, making it feasible for use with such high-speed applications as capillary gas chromatography. The interface and software for the IBM PC-compatible Peak Master system cost £2,500 (UK).

Aston Scientific Ltd has recently announced the introduction of Techni-Log, a PC add-on package for technical and **scientific data logging** (Reader Service No. 118). The £750 (UK) Techni-Log

package consists of a multi-channel analogue data acquisition interface, together with graphics display, analysis, curve-fitting and plotting software. With Techni-Log, data can be collected from a number of channels simultaneously, and displayed on the graphics screen. Sampling can be initiated on a time, event or threshold basis, and high and low alarm limits can be set. After sampling is completed, data can be manipulated using transform, statistical, plotting and curve-fitting routines, and can be saved on disk. Individual data files can be overlaid, subtracted and transformed, making the package useful in thermal analysis, spectroscopy and electrochemical techniques, says Astec.

The analytical tradition

Philips Analytical will have two major exhibitions at Labweek, one covering its analytical instrumentation, and another launching its new chromatography range. The model PU9800 **FTIR spectrometer** is one of the new products on the analytical side, and is the first Fourier transform infrared instrument to be produced by Philips (Reader Service No. 119). The FTIR spectrometer is controlled by a PC



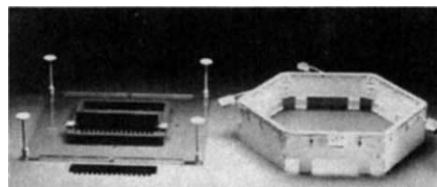
Philips Analytical launches FTIR spectrometry with the model PU9800.

AT computer operating Philips' Easy Access and Global Line Entry (EAGLE) infrared software, which also processes and manipulates data from the spectrometer. Philips says the model PU9800 is designed to fit the needs of both routine multi-use laboratories and research laboratories.

Ciba Corning has developed a package of equipment and reagents, named the Aminochrome system, for the complete **analysis of amino acids** from hydrolysis to quantitation (Reader Service No. 120). Because it uses gas-phase reagents for hydrolysis, Ciba Corning says Aminochrome does not exhibit the high levels of background contamination associated with liquid-phase hydrolysis. In the

Aminochrome system, free amino acids liberated by the hydrolysis are derivatized with dabsyl chloride, producing distinctive yellow chromophores which are stable for over four weeks at room temperature, according to the company. Ciba Corning claims that femtomole sensitivity is possible by absorption detection in the visible range, and that more sensitive measurements can be obtained with a photothermal refraction detector.

Pharmacia LKB has recently announced the introduction of its **hexagonal electrode kit** for use with its Pulsaphor pulsed field



Pharmacia LKB's hexagonal electrophoresis setup.

gel electrophoresis unit (Reader Service No. 121). Pharmacia LKB says the hexagonal electrode kit provides straight lane separations of large DNA molecules without the flaring associated with most pulsed field electrophoresis applications. The kit includes an hexagonal array of 20 optimally positioned electrodes, a 15×15 cm UV-transparent gel tray, a 15×15 cm casting frame and two 16-well combs.

Amersham International now supplies a range of **coloured protein molecular weight markers** for use in gel electrophoresis (Reader Service No. 122). The Rainbow series of markers are available in ^{14}C -methylated and non-radioactive forms. The radioactive markers allow the molecular weight of the protein to be accurately estimated following autoradiography. For less specific approximations, the molecular weight can be estimated from the leading edge of the non-radioactive coloured markers. Amersham says the Rainbow markers allow instant and unambiguous visualization of protein separation, and that low molecular weight markers can be run off the gel without compromising the identity of other bands. All of the protein marker series can also be transferred onto nitrocellulose or nylon membranes for Western blots: the colour marker can be used to check that transfer is complete. The Rainbow markers are available covering molecular weight ranges of 14,300–200,000 and 2,350–46,000. □

These notes are compiled by Carol Ezzell from information provided by the manufacturers. To obtain further details about these products, use the reader service card bound inside the journal. Prices quoted are sometimes nominal, and apply only within the country indicated.

Foreign support for US education

Richard Pearson

The increase in numbers of foreign students taking up postgraduate places in the United States is compensating for declining demand from US citizens.

UNTIL the mid 1970s the US higher education system had been experiencing continuous and rapid expansion, driven by the growing numbers of young people in the population, increasing participation by women in higher education and growing job opportunities for graduates, especially for those in science and engineering arising from the space programme. Between 1960 and 1974 the number of bachelors degrees awarded grew by 150% and the number of doctorates by over 200%. Although the numbers of bachelors degrees thereafter stabilized, the numbers of doctorates fell back by 10% in the 1970s before also stabilizing. The number of science degrees showed a broadly similar pattern, but the 1970s saw a significant 30% decline in the number of engineering doctorates awarded before they recovered in the mid 1980s.

This recovery, however, obscured a significant decline in the numbers of US citizens taking doctorates, a trend attributed to declining job prospects in academic life and the space and other federal programmes, this downturn being hidden by the rapid growth in overseas students. The output of US male doctorates fell by more than a half between 1970 and 1987, and although the number of US women studying grew rapidly, they still only accounted for one in four doctorates in 1986.

This decline in the numbers of US doctorates is causing great concern in the US, particularly if it continues in the future as it will then be exacerbated by the declining numbers of young people available to enter higher education over the next five years, and potentially even further by the current swing by students away from bachelors courses in technology and towards business studies (*Nature* 329, 90; 1987).

The problem has been obscured for some years by the massive compensating growth in the number of postgraduates coming from overseas. By the mid 1980s they were accounting for over half of all the doctorates awarded in engineering, including 65% of those in civil engineering and almost 60% in electronics engineering; representation was over 20% in the sciences with a high of around 40% in mathematics and computing but 14% in biological sciences (see table). At post-doctoral level their representation is even higher, 67% in engineering and 33% in the sciences, proportions that have also been growing.

Asia is the dominant area of origin accounting for over half of the foreign students, with China, India and Korea the leading countries, although the importance of the Middle East has declined in the 1980s. Although 80% are funded primarily by their home country or sources outside the US, many intend to, and do stay on in the US: 57% of engineers on temporary visas expect to stay, as do 46% of scientists, numbers that are well up on those of the early 1970s (31 and 28% respectively). A further one in four intend to continue with their studies in the US. They represent a major flow into the US high technology labour force: for example, by 1982 one in three engineering and computer science PhD entrants to the US labour market were foreign nationals. Many of these subsequently become

The percentage of foreign recipients of PhDs in 1985.

All science and engineering	28
Physical sciences	27
Mathematics	43
Computer science	37
Biological science	14
Chemical engineering	49
Civil engineering	67
Electronic engineering	58
Mechanical engineering	60

Source: *Foreign Citizens in US Science and Engineering*, NSF, 1987.

naturalized US citizens and their representation in the US workforce has grown dramatically; in 1982 they accounted for 13% of all scientists and engineers in the US workforce, 5% more than the previous decade. Over the same period the proportion on temporary visas was broadly static.

It has been argued that this high representation of foreign students has been depriving US citizens of postgraduate places and distorting the process and content of postgraduate education, but the evidence is to the contrary. In terms of student demand 86% of departments in engineering in a recent study (Boon or Bane, Institute for International Education, 1988) reported a shortage of US applicants, with few department heads expecting any improvement in the next few years. They report a continuing high demand from overseas students even though much of the available financial support is directed to US students. This demand from foreign nationals has been necessary to keep some of the programmes

open and it has only been in the more prestigious institutions that they have sought to, and been able to, restrict the number of foreign students. Although many faculty members reported that they spent more time advising and assisting foreign students, few said they adjusted the content and subject matter of the teaching to the needs of foreign students. On a qualitative level, many reported that they enhanced the quality of the student body and that the foreign students were more theoretically inclined than the US students; they were, however, less practical. Because of the shortfall of US postgraduates, foreign postgraduates play an essential role as teaching and research assistants although language and cultural differences could cause problems with US students. They were, however, often a positive factor when working with the large number of foreign postgraduates. On the negative side about 10% of departments said that their research activities were restricted in relation to defence contracts because only foreign and not US nationals were available.

At faculty level, shortages have been prevalent in many engineering and related subjects because of the earlier downturn in the throughput of postgraduate students. Here again foreign-born nationals play a key role, currently filling nearly one in three engineering faculty posts. They are on average younger than their US counterparts, probably reflecting their more recent recruitment, and they are also more research intensive in their activities. Although communication was once again a problem with the US students there were advantages when teaching the foreign students.

Overall the assessment on foreign participation is positive: in times of weak student demand they do not displace US students. Although there are some problems, they have a key role in propping up major areas of US postgraduate education both in research and as faculty, and will continue to be needed unless there is a massive and unexpected swing by US students in the next few years towards scientific and engineering subjects, a swing that must be large enough to counter the demographic downturn □

Richard Pearson is at the Institute of Manpower Studies, Mantell Building, University of Sussex, Brighton BN1 9RF, UK.